The Susceptibility of Juvenile Chum Salmon (*Oncorhynchus keta*) to Predation Following Sublethal Exposure to Elevated Temperature and Dissolved Gas Supersaturation in Seawater

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#### THE SUSCEPTIBILITY OF JUVENILE CHUM SALMON (ONCORHYNCHUS KETA) TO PREDATION FOLLOWING SUBLETHAL EXPOSURE TO ELEVATED TEMPERATURE AND DISSOLVED GAS SUPERSATURATION IN SEAWATER

by

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#### PREFACE

This report is one in a series that describe the results of studies on the effects of heated seawater on juvenile chum salmon (*Oncorhynchus keta*). The studies were initiated in response to concerns regarding the potential effects on salmon due to the thermal discharge from British Columbia Hydro and Power Authority's (BC Hydro) Burrard Generating Station, into the marine waters of Port Moody Arm, Burrard Inlet, BC. This gas-fired steam electric generating station operates under a permit from the provincial government, and utilizes a once-through seawater cooling system. The permit allows for the discharge of up to 1.7 million cubic metres daily of cooling waters ( $\leq$  27 °C), drawn from, and discharged to, Port Moody Arm. An environmental impact study to assess any effects due to the thermal discharge was a requirement of an amendment to the provincial permit. An environmental assessment study plan was submitted by BC Hydro to federal and provincial regulatory authorities in 1996, and it was approved in 1997.

The Department of Fisheries and Oceans entered into a co-operative research venture with BC Hydro on selected aspects of the environmental assessment. Other studies investigated the effects of the thermal effluent on the growth of juvenile chum salmon, the heat budget of Port Moody Arm and the thermal input from mud flats, an assessment of the potential effects of the effluent on migrating and resident fish, and the potential effects on planktonic organisms drawn into the plant and those entrained in the thermal effluent plume.

The Department of Fisheries and Oceans undertook two studies in 1997. The behavior of chum salmon was examined under conditions that mimicked those that the fish may encounter in Port Moody Arm during a changing thermal regime and under thermally-stratified conditions. The response of the fish to food, their swimming, and school positions, were quantified in relation to the experimental conditions. In the second study, 6 m-deep "preference-avoidance" cages were used in Port Moody Arm to examine the vertical distribution of chum salmon in relation to the discharge of heated cooling water. The results were related to the ambient aquatic conditions to reveal differences or similarities in the vertical distribution of salmon with proximity to the discharge location, and to identify variables that accounted for these changes. These studies revealed that juvenile salmon will use waters that are potentially lethal to them in order to feed and that they will occupy surface waters in the wild in which temperatures exceeded laboratory-derived avoidance thresholds and which also contained supersaturated levels of dissolved gases.

The foregoing studies led us to examine whether the fitness and performance of juvenile chum salmon would be affected by exposure to the more extreme conditions recorded in Port Moody Arm in 1997. Because juvenile salmon are subjected to natural predation pressure during their early sea life, and since health and fitness are essential requirements for survival, we assessed the vulnerability of chum salmon to predation following 48-h, 24-h, and 12-h exposure to seawater at 20.7 °C and 115%, 120% and 130% total gas pressure, respectively. Copper and quillback rockfish, kelp greenling, and Pacific staghorn sculpin were the predators, which were returned to the wild after experimentation. This report describes the main results of the study. The behavior of the rockfish predators and their chum salmon prey are presented in another report.

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#### ABSTRACT

#### Birtwell, I.K., J.S. Korstrom, M. Komatsu, B.J. Fink, L.I. Richmond, and R.P. Fink. 2001. The susceptibility of juvenile chum salmon (*Oncorhynchus keta*) to predation following sublethal exposure to elevated temperature and dissolved gas supersaturation in seawater. Can. Tech. Rep. Fish. Aquat. Sci. 2343: 149 p.

The effects of sublethal exposure to a combination of elevated temperature and dissolved gas supersaturation [an elevation in total gas pressure (TGP)] on the susceptibility of juvenile chum salmon (*Oncorhynchus keta*) to predation in seawater was assessed in laboratory experiments carried out between May and August 1998.

The experimental treatment exposed the fish to a progressive rise in temperature from ambient (11 °C) to 20.7 ± 0.2 °C and TGP levels of 115% (T+115), 120% (T+120), or 130% (T+130) for 48 h, 24 h, and 12 h respectively. Thereafter the temperature was decreased over a  $37 \pm 7$  min period to that of ambient seawater. The thermal and TGP treatment levels were chosen to encompass, and also exceed, values to which juvenile chum salmon may be exposed during summer in the Port Moody Arm of Burrard Inlet, BC. In addition to natural thermal inputs to these waters, an electric generating station discharges up to 1.7 million cubic meters of heated cooling waters ( $\leq$  27 °C) daily. The duration of exposure of the chum salmon to the experimental conditions was relatively brief considering the propensity of the species to reside in shallow surface waters for days to weeks during the early phase of their life in marine waters. However, the conditions were expected to be at sublethal levels and stress the treated fish. During the exposures, 99.9%, 98.9%, 97.9%, and 89.8% of fish survived in the control groups and those in the T+115, T+120, and T+130 treatment groups, respectively.

A population comprising 20 individuals of similar size from each of the exposed and their respective control groups was then challenged to survive predation by piscivorous marine fish in 11 °C air-equilibrated seawater. At the same time, samples of fish were removed from each treatment and control group and, together with the few fish that had died during the exposure period, examined for signs of Gas Bubble Trauma (GBT) which can occur as a result of exposure to elevated levels of TGP.

Significant differences in the prevalence and severity of signs of GBT for the control group versus each of the treated groups were found in the eyes, lateral line, caudal fin, and gills of fish. These differences were evident for live fish as well as for those that died during the exposures (T+130 treatment only).

The incidence of gas emboli in the gills was 100% in the few fish that died during the 12-h exposure to the T+130 treatment. These dead fish also had the highest incidence of emboli in the lateral line (44.3%) and sub-cutaneous emphysema in the caudal fin (65.6%). There was no incidence of gas emboli in the gills of fish that survived the exposures to warm seawater and dissolved gas supersaturation.

The incidence of emboli and hemorrhage in the lateral line, and emphysema and hemorrhage in the caudal fin of treated fish was significantly elevated and different from that of the controls, whereas the incidence of exopthalmia was only significantly different from that of the controls in the T+120 and T+130 treatment groups.

Forty-five predator challenge tests, each of 60 minutes duration, were carried out in shallow (41-cm deep) annular 1600-L raceways using kelp greenling (*Hexagrammus decagrammus*) and Pacific staghorn sculpin (*Leptocottus armatus*) predators.

Eighteen predator challenge tests of  $\leq$  90 minutes duration were carried out in a 2.4-m deep, 4500-L Water Column Simulator (WCS) in which copper rockfish (*Sebastes caurinus*) and quillback rockfish (*Sebastes maliger*) were the predators. All the predatory fish survived capture, handling, and residency at the West Vancouver Laboratory, and they were returned to the wild at the conclusion of the experiments.

In the shallow water raceways, more juvenile chum salmon that had been previously exposed to T+115, T+120, or T+130 treatments were consumed in eight, eleven, and ten of the respective fifteen tests, relative to those consumed from the control groups. However, the difference in the consumption of treated versus control fish was only significant in those tests in which the chum salmon had been previously exposed to either the T+120 or the T+130 treatment (9.8% and 10.7%). The difference in the consumption of treated versus control fish was 0.3% in those tests in which the chum salmon had been previously exposed to the T+115 treatment.

In the deep-water WCS challenge tests, more juvenile chum salmon that had been previously exposed to T+115, T+120, or T+130 treatments were consumed in four, six, and three of the respective six tests relative to those from the control groups. However, the difference in the consumption of treated versus control fish was only significant for those tests that used chum salmon which had been previously exposed to the T+120 treatment (22.8% more treated fish consumed). The difference in the consumption of treated versus control fish was 6.5% and 11.3% for those tests that used chum salmon which had been previously exposed to the T+130 treatment, respectively.

Irrespective of the pre-treatment of the chum salmon, the treated fish were more susceptible to predation than were the controls and, the rockfish preferentially attacked solitary fish (63% of all attacks) rather than the school. This preferential choice to attack solitary fish rather than the school was not significant when the first five minutes of each data set was removed, implying that most selective predation on solitary fish occurred within this time.

The rockfish were also more successful in capturing chum salmon from populations comprising control fish and those that had been previously exposed to T+120 or T+130 treatments than from populations of control fish and those previously exposed to the T+115 treatment. That is, there was a lower number of attacks, (10.3, and 10.7 attacks per predator

versus 14.6, respectively), and a greater percentage of captures per attack, (31.8% and 30.0%, versus 17.0% respectively).

The efficiency with which the rockfish captured the chum salmon prey (expressed as a ratio of the number of successful captures to the number of attacks) was highest in those tests in which the treated fish had been previously exposed to T+120 or T+130 treatments. The respective capture to attack ratios were 1:5.8, 1:3.1 and 1:3.3, for the T+115, T+120 or T+130 treatment groups.

These quantitative data reveal the significantly enhanced vulnerability of juvenile chum salmon to predation following exposure to the combined sublethal stressors of warm (20.7 °C) seawater and elevations of TGP of 120% for 24 h, or 130% TGP for 12 h. The results indicate that the performance of the juvenile salmon was compromised by the treatments they received, thereby rendering them more susceptible to predation. We speculate that this increased susceptibility to predation was most likely associated with a performance deficit due to the effects of gas bubbles reducing the sensory capabilities and blood circulation of the fish. Increased vulnerability to predation could have occurred because of the debilitating effects of elevated temperature and TGP on swim performance and an associated reduced ability to recover from fatigue brought on by predator attacks and the need for repetitive escape responses.

The numbers of juvenile chum salmon typically decline in near shore coastal areas during the late spring and early summer. At this time, surface water temperatures and dissolved gases in Port Moody Arm tend to increase to levels that, in combination, could lead to an increased vulnerability of juvenile chum salmon to predation and especially so with prolonged (>48 h) residence in these waters. The innate tendency of these juvenile salmon to reside for days to weeks in the uppermost surface waters during their early marine life potentially increases the risk of exposure to elevated temperatures and TGP in Port Moody Arm. We deduce, however, that such a risk would be restricted to few individuals of the chum salmon population that utilize these waters during the late spring and early summer. Therefore, the additional and indiscriminate effect of exposure to the combination of these variables to increase the mortality of the chum salmon population through predation, over that which occurs naturally, will probably be minimal, and restricted to a small fraction of the population.

**KEY WORDS**: predation, predator-prey, chum salmon, rockfish, sculpin, greenling, temperature, Total Gas Pressure, combined stressors.

#### RÉSUMÉ

Birtwell, I.K., J.S. Korstrom, M. Komatsu, B.J. Fink, L.I. Richmond, and R.P. Fink. 2001. The susceptibility of juvenile chum salmon (*Oncorhynchus keta*) to predation following sublethal exposure to elevated temperature and dissolved gas supersaturation in seawater. Can. Tech. Rep. Fish. Aquat. Sci. 2343: 149 p.

Les effets d'une exposition sublétale à une température élevée combinée à une sursaturation en gaz dissous [élévation de la pression totale du mélange gazeux (PTG)] sur la vulnérabilité de kétas juvéniles (*Oncorhynchus keta*) à la prédation en mer ont été évalués dans le cadre d'expériences de laboratoire menées de mai à août 1998.

Dans le traitement expérimental, les poissons ont été exposés à une élévation graduelle de la température ambiante (11 °C) à 20.7 °C  $\pm$  0.2 °C, et de la PTG de 115% (T+115), 120% (T+120) ou 130% (T+130) pendant respectivement 48 h, 24 h et 12 h. Par la suite, la température de l'eau a été ramenée à la température ambiante de l'eau de mer sur une période de 37  $\pm$  7 minutes. Les conditions expérimentales de température et de PTG ont été choisies de manière à englober, et même à dépasser, celles auxquelles les kétas juvéniles peuvent être exposés pendant l'été dans le bras Port Moody de l'inlet Burrard (C-B). En plus des apports thermiques naturels, une centrale électrique rejette jusqu'à 1.7 million de mètres cubes d'eau de refroidissement chauffée ( $\leq$  27 C) par jour. La durée d'exposition des kétas aux conditions expérimentales a été assez courte compte tenu de la tendance de l'espèce à demeurer dans les eaux de surface peu profondes pendant des jours, voire des semaines, au début de leur vie en mer. Toutefois, les conditions étaient supposées être sublétales et exercer un stress sur le poisson qui y est exposé. Pendant les expériences, respectivement 99.9%, 98.9%, 97.9% et 89.8% des poissons ont survécu dans les groupes témoins et dans les groupes soumis aux traitements T+115, T+120 et T+130.

Une population de 20 individus de taille similaire provenant chacun des groupes exposés au traitement et de leur groupe témoin respectif ont ensuite été soumis à une expérience de survie à la prédation exercée par des poissons marins piscivores en eau de mer à température de 11 C équilibrée à celle de l'air. Parallèlement, on a prélevé des échantillons de poissons dans chaque groupe expérimental et dans chaque groupe témoin, auxquels on a ajouté les quelques poissons qui sont morts pendant la période d'exposition, pour rechercher des signes d'embolie gazeuse, qui peut survenir à la suite d'une exposition à des PTG élevées.

Des différences importantes de prévalence et de gravité des signes d'embolie gazeuse dans le groupe témoin ont été observées au niveau de l'oeil, de la ligne latérale, de la nageoire caudale et des branchies par rapport aux groupes expérimentaux. Elles étaient évidentes chez les poissons vivants ainsi que chez ceux qui sont morts pendant les expositions.

Tous les poissons qui sont morts (100%) pendant l'exposition de 12-h à une PTG de T+130 présentaient des signes d'embolie gazeuse dans les branchies. L'incidence la plus élevée

d'embolie dans la ligne latérale (44.3%) et d'emphysème sous-cutané de la nageoire caudale (65.6%) a aussi été observée chez les poissons morts. Aucun cas d'embolie gazeuse n'a été relevé dans les branchies des poissons qui ont survécu aux expositions à l'eau de mer chaude et à une sursaturation en gaz dissous.

Chez les poissons exposés au traitement, l'incidence d'embolie et d'hémorragie au niveau de la ligne latérale, d'emphysème et d'hémorragie dans la nageoire caudale était significativement élevée et différente de celle relevée chez les témoins, tandis que l'incidence de l'exophtalmie différait de façon significative seulement dans les groupes T+120 et T+130 par rapport aux témoins.

Quinze tests d'exposition à des prédateurs, d'une durée de 60 minutes chacun, ont été menés dans des bassins annulaires (1600-L) peu profonds (41-cm de profondeur) en utilisant comme prédateurs le sourcil de varech (*Hexagrammus decagrammus*) et le chabot armé (*Leptocottus armatus*). Six autres tests d'une durée  $\leq$  90 minutes ont été effectués dans un simulateur de la colonne d'eau de 4500 L (2.4-m de profondeur) dans lequel le sébaste cuivré (*Sebastes caurinus*) et le sébaste à dos épineux (*Sebastes maliger*) ont servi de prédateurs. Tous les poissons prédateurs ont survécu à la capture, à la manutention et au séjour au Laboratoire de Vancouver ouest, et ils ont été retournés dans leur milieu naturel à la fin de l'expérience.

Dans les bassins peu profonds, les kétas juvéniles qui avaient été exposés aux traitements T+115, T+120 ou T+130 ont été plus nombreux à être ingérés dans huit, onze et dix des quinze tests respectifs par rapport à ceux qui ont été consommés dans les groupes témoins. Toutefois, les différences de consommation des poissons expérimentaux par rapport aux poissons témoins n'étaient significatives que pour les tests dans lesquels les kétas avaient été exposés précédemment aux traitements T+120 ou T+130; les poissons exposés au traitement étaient plus vulnérables à la prédation que les poissons témoins.

Dans les tests en bassin profond, les kétas juvéniles exposés aux traitements T+115, T+120 ou T+130 ont été plus nombreux à être consommés dans quatre, six, et trois des six tests respectifs par rapport à ceux qui ont été ingérés dans les groupes témoins. Toutefois, les différences de consommation des poissons expérimentaux par rapport aux témoins n'étaient significatives que pour les tests qui utilisaient des kétas exposés au préalable au traitement T+120.

Abstraction faite du pré-traitement des kétas, les poissons traités étaient plus vulnérables à la prédation que les poissons témoins, et les sébastes attaquaient de préférence les poissons solitaires (63% de toutes les attaques) plutôt que des bancs de poissons. Cette attaque préférentielle des poissons solitaires plutôt que des bancs de poissons n'était pas significative lorsqu'on éliminait les cinq premières minutes de chaque série de données, ce qui signifie que la prédation sélective sur les poissons solitaires s'exerçait principalement pendant cette période. Les sébastes réussissaient mieux à capturer des kétas dans les populations comprenant des témoins et des poissons exposés au préalable aux traitements T+120 ou T+130 que dans les

populations composées de témoins et de poissons exposés au traitement T+115. Cela signifie qu'il y a eu moins d'attaques (respectivement 10.3 et 10.7 attaques par prédateur contre 14.6) et un pourcentage plus élevé de capture par attaque (respectivement 31.8% et 30.0% contre 17.0%).

L'efficacité avec laquelle les sébastes capturent les kétas (exprimée sous forme de rapport entre le nombre de captures réussies et le nombre d'attaques) était la plus élevée dans les tests dans lesquels le poisson traité avait été exposé aux traitements T+120 ou T+130. Les rapports respectifs entre les captures et les attaques étaient de 1:5,8, 1:3,1 et 1:3,3 pour les groupes exposés aux traitements T+115, T+120 ou T+130.

Ces données quantitatives montrent que la vulnérabilité des kétas juvéniles à la prédation est nettement accrue à la suite d'une exposition à une combinaison d'agents stressants sublétaux: eau chaude (20.7 C) et augmentation de la PTG de 120% pendant 24 h, ou de 130% pendant 12 h. Les résultats révèlent que les traitements auxquels ont été exposés les saumons juvéniles ont nui à leur performance, ce qui les a rendus plus vulnérables à la prédation. Nous émettons l'hypothèse que cette vulnérabilité accrue à la prédation serait probablement associée à une perte de performance attribuable aux effets des bulles de gaz qui réduisent les capacités sensorielles et la circulation sanguine chez le poisson. L'accroissement de la vulnérabilité à la prédation pourrait être attribuable aux effets débilitants d'une élévation de la température et de la PTG sur la performance natatoire et d'une réduction connexe de la capacité à récupérer de la fatigue provoquée par les attaques des prédateurs et par la nécessité des réactions de fuite à répétition.

En général, le nombre de kétas juvéniles diminue dans les zones littorales à la fin du printemps et au début de l'été. À ce moment-là, la température de l'eau de surface et les gaz dissous dans le bras Port Moody ont tendance à augmenter pour atteindre des valeurs, qui, combinées, pourraient entraîner une plus grande vulnérabilité à la prédation des kétas juvéniles, tout particulièrement dans le cas d'un séjour prolongé (>48 h) dans ces eaux. La tendance naturelle de ces juvéniles à demeurer pendant des jours, voire des semaines, dans la couche supérieure des eaux de surface au début de leur vie en mer peut augmenter le risque d'exposition à une température et une PTG élevées dans le bras Port Moody. Nous concluons, cependant, qu'un tel risque ne toucherait que quelques individus de la population de kétas qui fréquentent ces eaux à la fin du printemps et au début de l'été. Donc, le risque global supplémentaire de voir une exposition à la combinaison de ces variables accroître la mortalité par prédation dans la population de kétas, par rapport à la mortalité naturelle, sera probablement négligeable et limité à une petite proportion de la population.

**MOTS-CLÉS:** prédation, prédateur-proie, kéta, sébaste, chabot, sourcil, température, pression totale du mélange gazeux, agents stressants combinés.

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#### INTRODUCTION

## BURRARD GENERATING STATION (BGS) AND ENVIRONMENTAL IMPACT STUDIES

The study reported herein was initiated in relation to potential increases in the thermal discharge from British Columbia Hydro and Power Authority's (BC Hydro's) Burrard Generating Station (BGS), into the marine waters of Port Moody Arm, Burrard Inlet, BC. This gas-fired steam-electric station operates under a permit from the provincial government, and utilizes a once-through seawater cooling system. The permit allows for the discharge of up to 1.7 million cubic metres daily of heated ( $\leq$  27 °C) cooling water, drawn from, and discharged to, Port Moody Arm [a 6.5-km long inlet with an average depth of 8.8 m at low tide (Waldichuk 1965)]. An environmental study to assess any effects due to the thermal discharge was a requirement of an amendment to the provincial permit. Federal and provincial government regulatory authorities considered it important to examine the effects of the thermal discharge from the BGS. Accordingly, BC Hvdro submitted a plan to these authorities in December 1996 that identified a number of studies to be initiated in 1997 as part of a 2-year environmental assessment (BC Hydro 1996). The study plan was approved in 1997 and resulted in research on the effects of the thermal discharge on the growth (Greenbank et al. 1998, 2001) and behavior (Birtwell et al. 1998, 2001; Korstrom et al. 1998) of chum salmon, the potential effect on planktonic organisms (Duval 1998), the species of fish and their distribution in Port Moody Arm (Aquatic Resources Ltd. 1998), and the heat budget for these waters (ASL Environmental Sciences 1998).

# PREVIOUS LABORATORY AND FIELD STUDIES ON THE BEHAVIOR OF CHUM SALMON

In 1997, a laboratory technique was used to examine the behavior of juvenile chum salmon (*Oncorhynchus keta*) to thermal change (Birtwell et al. 2001; Korstrom et al. 1998). Chum salmon were chosen as the test species due to their abundance and prevalence (March to August) in the contiguous waters of Burrard Inlet (Nelles 1978; Macdonald and Chang 1993). Spawning populations occur in local streams and rivers, and juveniles from these fish will also utilize the waters of Port Moody Arm and may encounter the thermal plume associated with the heated cooling water discharged by the BGS. In the laboratory studies the juvenile chum salmon preferred temperatures between 13.7 °C and 17.9 °C, and increasing temperatures resulted in increasing avoidance. However, the presence of food encouraged them to feed in waters at temperatures that were potentially lethal to them with prolonged exposure (Birtwell et al. 2001; Korstrom et al. 1998). To examine whether these results (which were obtained in the relatively sensory-deprived environment of the laboratory) would be applicable to the natural environment, we used an experimental technique to examine the vertical distribution of chum salmon in relation to the discharge of heated cooling water from the BGS, and the

additional natural heat input from the solar heating of mud flats and waters in Port Moody Arm (Birtwell et al. 1998). We determined that the vertical distribution of chum salmon was not affected significantly by the thermal discharge and, furthermore, that the chum salmon continued to occupy the waters close to the surface even when temperatures and dissolved gasses were at levels considered to be potentially stressful if not lethal with prolonged exposure (Birtwell et al. 1998). This result was not unexpected for it is apparent that even when a fish has the capability to detect change in its' environment, and to respond to that change, it has been shown that choices which we deem to be appropriate are not necessarily those which are made by fish (Birtwell and Kruzynski 1989).

Although the effects of transient excursions by juvenile chum salmon into potentially lethal conditions were not quantified in these laboratory and field experiments, other studies have determined that the choice of fish to occupy habitats in response to primary environmental (adaptive) cues can result in their debilitation and death in environments that have been impacted by anthropogenic activities. For example, the strength of the feeding response overrode typical avoidance responses to lethal temperatures and resulted in the death of fish in thermal gradients (Neill and Magnuson 1974; Munson et al. 1980); adult sockeye salmon occupied stressful and potentially lethal hypoxic waters because of a fidelity to waters with temperatures around 10 °C to 12 °C (Birtwell et al. 1994; Spohn et al. 1996) thus jeopardizing their health, performance, and survival (Birtwell et al. 1994; Farrell et al. 1998; Jain et al. 1998); and juvenile chinook salmon occupied inter-cobble habitats within the plume of treated (non-acutely toxic) pulp mill effluent (Emmett et al. 1996) potentially increasing their susceptibility to predation (Campbell et al. 1995; 1996). Marcello and Fairbanks (1974) reported that Atlantic menhaden (Brevoortia tyranus) were attracted to the thermally heated waters discharged from a nuclear power plant and that they died from exposure to elevated levels of total gas pressure (TGP). Although fish may avoid dissolved gas supersaturation (DGS), other factors may alter their response to this variable. Meldrim et al. (1973) noted that the golden shiner (Notemigonus crysoleucas) usually avoided gas supersaturation of 110%, but when temperature increases of 5 °C -10 °C were associated with the supersaturation, thermal preference overrode the avoidance response.

Whether the occupancy of the thermally-enhanced and dissolved gas-supersaturated surface waters of Port Moody Arm would be harmful to juvenile chum salmon required resolution as either one of these variables has the potential to harm fish given a sufficient exposure time and level. There was, however, no information available on the combined effects and significance of these variables at the sublethal level on juvenile chum salmon. To address this deficiency we designed the study reported herein to provide information on the effects of exposure of juvenile chum salmon to sublethal temperature and dissolved gas supersaturation (DGS) in seawater. The focus of the research was on the potential of a combination of these variables to compromise the performance and

behavior of chum salmon to the extent that their survival may be jeopardized through increased vulnerability to predation.

#### **CONSIDERATIONS OF TEST METHODOLOGY**

Although it would have been possible to study the combined effects of temperature and TGP on juvenile chum salmon through a series of laboratory assays that indicated effects within the organism (refer to Wedemeyer et al. 1991), a whole organism response was considered more appropriate. Furthermore, we required a test that had an end point that was not only relevant to the survival of the individual but also one which was ecologically meaningful. A fish that is less fit, and therefore is less able to effectively compete for food and avoid predators is, potentially, likely to be selectively preyed upon (Bams 1967). Accordingly, a series of predator challenge tests was chosen as a means to assess the effects of sublethal exposure to a combination of elevated DGS and temperature.

The utility of predator challenge tests has been demonstrated through numerous studies designed to assess the effects of, for example, heat (Coutant 1973; Sylvester 1973) and cold temperature shock (Coutant et al. 1974), TGP (Mesa and Warren 1997), toxicants (Brown et al. 1985; Schneider et al. 1980; Birtwell et al. 1994), differences in size of prey (Beall 1972; Taylor and McPhail 1985) disease (Herting and Witt 1967; Mesa et al. 1994), handling and transport (Sigismondi and Weber 1988; Mesa 1994), descaling (Gadomski et al. 1994), naive vs predator experienced or conditioned prey (Patten 1977; Olla and Davis 1989; Järvi 1990; Healey and Reinhardt 1995), fitness of fish from hatcheries (Bams 1967; Ginetz and Larkin 1976; Beall 1972), and osmotic stress (Järvi 1989a, b; Handeland et al. 1996). Coutant et al. (1979) and Mesa et al. (1994) provide reviews of the application of predator challenge tests. Such tests have generally proven to be more relevant to an assessment of the consequences of stress (Mesa 1994) than typical biochemical indices (e.g. corticosteroids) because the behaviors that have a high survival value tend to recover faster to baseline levels than do some biochemical indicators (Olla et al. 1992; Mesa 1994; Schreck 1981; Schreck et al. 1997), although this is not always so (Olla et al. 1995).

The success of predator challenge experiments depends on many factors including the appropriate selection and capture of the predators and their maintenance in conditions that promote their well-being and the continuation of behavioral (e.g. feeding) repertoires evident in nature. An interactive relationship between predator and prey is fundamental to the predator challenge experiment. In this study copper rockfish (*Sebastes caurinus*), quillback rockfish (*Sebastes maliger*), kelp greenling (*Hexagrammus decagrammus*), and Pacific staghorn sculpin (*Leptocottus armatus*), predators that would feed on chum salmon, were used.

The rockfish species typically occur in deeper near shore areas in the same species assemblage, often in close proximity and in complex habitat (Murie et al. 1994), although copper rockfish have less association with broken rock and kelp than does quillback rockfish (Richards 1987). Both species are flexible in their diet and feed extensively on fish at times (Murie 1995). They are crepuscular in feeding habit, that is, active in the hours of twilight or preceding dawn, but quillback rockfish primarily feed around mid-day (Murie 1995), and neither species feeds at night.

The kelp greenling are considered to be "trophic generalists" (Nemeth 1997a, b), as are sculpins (Norton 1991; Armstrong et al. 1995). Sculpin predation on salmonid fry in fresh water has been well documented (e.g. Hunter 1959; Patten 1971; Moyle 1977) and Pacific staghorn sculpins were documented to feed from dawn to dusk on juvenile chum salmon in the estuary of the Big Qualicum River (Mace 1983), and greenling on chum salmon in Japan (Nagasawa and Kaeriyama 1995).

The greenling and sculpins were captured close to shore and they were used together in predator challenge tests within shallow-water raceway apparatus. Similarly the rockfish that were captured in deeper waters, were used in apparatus [Water Column Simulator (WCS)] which enclosed a larger and deeper volume of water.

Clearly, death in the wild is an expected end point to an individual's life, and one would logically expect visible corpses from the death of all organisms were it not for the actions of predators and scavengers. Research has shown that fish can survive exposure to a range of potentially lethal compounds, effluents, and circumstances, but may be physiologically and behaviorally compromised as a consequence. Without knowledge of the potential consequences of such compromises to the survival of these fish when faced with the additional rigors associated with life in the wild, it would be easy to overlook indirect mortality or "ecological death" (Sprague 1971; Coutant et al. 1979; Kruzynski and Birtwell 1994; Mesa et al. 1994). That few dead organisms are visible at times other than those in which catastrophic events occur is testimony to the natural consumption and assimilation of organisms, and the fitness and fortunes of others. Because predation is a major source of juvenile salmon mortality during their early marine life (Parker 1968; Bax 1983; Mace 1983; Healey 1982) and that predators generally attack prey in substandard or unusual condition (Neill and Cullen 1974; Coutant et al. 1979; Landeau and Terborgh 1986; Temple 1987; Gadomski and Hall-Griswold 1992; Kruzynski and Birtwell 1994; Mesa and Warren 1997), we considered that the use of predator challenge tests had merit in not only assisting in the elucidation of the effects of temperature and supersaturated TGP on juvenile chum salmon, but also in providing a potentially highly relevant and ecologically meaningful result.

#### MATERIALS AND METHODS

#### CHUM SALMON TRANSPORT AND MAINTENANCE

The stock of juvenile chum salmon used in these experiments was obtained from Indian River (North Vancouver, BC) brood stock on November 5, 1997. The eggs were incubated at the Seymour River Hatchery (North Vancouver, BC) and the fry were ponded on April 21, 1998. On April 27, 1998, 7800 chum salmon (mean ± SD; weight  $0.44 \pm 0.07$  g, fork length  $39 \pm 2$  mm) were transported from the hatchery to seawater holding facilities at the Department of Fisheries and Oceans (DFO) West Vancouver Laboratory (WVL). The transport of fish from the hatchery occurred when fry would be entering salt water in the wild. Typically, chum salmon fry emerge from the gravel and promptly migrate downstream to estuarine waters at a size of 30 - 40 mm (Healey 1982; Salo 1991). Transport was accomplished within 1 h using a truck fitted with a 500-L insulated plastic tank supplied with compressed air from a portable compressor unit. A mesh bag containing 500 g Ammonex<sup>™</sup> (Argent Chemical Laboratories, Redmond, WA), a natural clay that rapidly binds to, and eliminates ammonia, was placed in the transport tank to prevent the accumulation of this waste metabolite. To reduce osmotic stress during the rapid transition to seawater from the fresh water hatchery environment (7.5 °C), the transport tank contained an approximately isosmotic saline solution (salinity 9 ‰) at a temperature of 8.2 °C.

Upon arrival at the laboratory, the fish were vaccinated against *Vibrio* sp. via an immersion bath technique with BIOVAX 1300<sup>TM</sup> (Alpharma, Bellevue, WA), a prophylactic health management tool. *Vibrio* sp. are opportunistic, facultative, bacterial pathogens of fish which are ubiquitous in marine and estuarine environments. Fish reared in fresh water are not prepared immunologically to cope with *Vibrio* sp. infections and chum and pink salmon are especially susceptible to the disease (Warren 1981). BIOVAX 1300<sup>TM</sup> is a water based, whole cell bacterin formulated from killed *Vibrio anguillarum* serotype 1 and *Vibrio* sp. and, typically, natural contact sustains immunity. The bacterin suspension was diluted in the transport tank water (1:100). The population of juvenile chum was exposed to this vaccine for 1h, during which time the dissolved oxygen concentration of the water in the holding tank was kept at saturation using compressed air delivered through air stones. Following vaccination the salinity was increased to 16 ‰ over 1 h by the addition of seawater at 28 ‰. The fish were then transferred, using a low abrasion, knotless nylon dip net, to a 6200-L outdoor holding tank.

Determinations of water quality variables were made daily in all stock tanks to ensure the prompt recognition of any potential problems and expedite the implementation of mitigative measures. The stock tank was continuously supplied with air-equilibrated seawater (salinity  $28.3 \pm 0.7$  %, temperature  $11.0 \pm 1.0$  °C, dissolved oxygen  $92 \pm 6\%$  of

air saturation, TGP 99.2  $\pm$  0.9%, pH 7.7  $\pm$  0.1). Seawater at the laboratory was sand filtered, after being pumped from an intake structure situated approximately 100 m offshore at a depth of 15 m below the low tide level. Water flow was delivered to the tank at approximately 60 L·min<sup>-1</sup> which ensured a 90% replacement within 4 h (Sprague 1969). Fish density was maintained at  $\leq$  3.8 kg·m<sup>-3</sup>, and flow-loading density at  $\leq$  0.5 kg·L<sup>-1</sup>·min<sup>-1</sup>. An air stone, connected to an oil-free, single stage, direct drive, electric air compressor (Coleman Powermate Compressors Inc., Springfield, MN) provided additional aeration (a precautionary measure implemented to protect the fish in the event of a water flow disruption).

Stock fish were fed a maintenance ration (Moore Clarke, Vancouver, BC, Nutra Fry<sup>TM</sup>, crumble #0, #1, and #2) at between (1.2% - 4.3%) wet body weight d<sup>-1</sup> which resulted in a Body Condition Index (BCI) of  $0.87 \pm 0.05$ . The ration was calculated from feed tables supplied by Moore Clarke, (Vancouver, BC), and represented 0.5 to 0.75 of the ration expected to yield optimal growth. This ration was provided in an attempt to control fish growth rate so that they would be at an appropriate size for the predators during the experimental period. Food delivery to the tank was via two automatic belt feeders (Zeigler Bros. Ltd., Gardner, PA), situated on opposite sides of the tank, and set for continuous operation from dawn to dusk. This feeding regime attempted to avoid behavioral entrainment of the fish on a set feeding schedule, reduce aggression, and to ensure a more equal dispersal of food to all fish to minimize size variability in the population. The salmon were held under a natural photoperiod and were acclimated for a period of at least seven weeks prior to experimentation. To exclude avian predators, the tank was covered with 4-cm diameter plastic (vexar) mesh which was overlain with a woven black polyethylene sheet to provide shade and minimize visual disturbance.

Despite vaccination, an epizootic of *Vibrio* sp. became prevalent in the stock tank fish after 15 weeks of holding at the WVL, and experiments were curtailed for two weeks. The stock fish were effectively treated with the antibiotic Oxytetracycline<sup>TM</sup> (Syndel Laboratories Ltd., Vancouver, BC) at a rate of 100 mg·kg<sup>-1</sup>d<sup>-1</sup>. The fish were hand fed over a 10-d treatment period and the drug was administered by incorporating it into the feed ration via a topical coating of canola oil. Prior to, and directly following the drug therapy, mortality in the stock tank was negligible.

The stock of chum salmon were found to have eye opacities which ranged in degree of occlusion of the eye (96.2% of control fish, and 85.6% of all treated fish had  $\leq 10\%$  eye opacity). While this condition was not severe, we had samples of the fish examined by a veterinarian to ascertain the possible cause of the opacity. It was deduced that the condition was associated with an early transfer to seawater and not a dietary deficiency which can also lead to opacities and cataracts in the eyes of juvenile salmon (Richardson et al. 1986). These fish were fed a high quality diet at a ration that ensured suitable growth and health as evidenced by the mean (0.87) Body Condition Index (BCI) for juvenile chum salmon. This BCI value compares favorably with that of chum salmon

used in earlier experiments (Birtwell et al. 2001) wherein the fish had been fed a ration of 3% - 8% wet body weight·d<sup>-1</sup>, and the determinations by Davis and Olla (1987) for chum salmon fed 7.2% wet body weight·d<sup>-1</sup> (BCI, 0.82).

#### PREDATOR CAPTURE AND MAINTENANCE

Concern over the wellbeing and treatment of the predators while in captivity was expressed by the DFO Animal Care Committee. Compliance with their requirements was a necessary step in order to obtain approval to proceed with these studies.

The sizes of the predators used in experiments were: copper rockfish (*Sebastes caurinus*, weight 490 g, fork length 29.5 cm, n=1), quillback rockfish (*Sebastes maliger*, weight  $575 \pm 116$  g, fork length  $31.6 \pm 2.5$  cm, n = 5), kelp greenling (*Hexagrammus decagrammus*, weight  $652 \pm 294$  g, fork length  $35.0 \pm 4.7$  cm, n = 12) and Pacific staghorn sculpin (*Leptocottus armatus*, weight  $410 \pm 16$  g, fork length  $29.3 \pm 0.4$  cm, n = 2). Predators were obtained by angling in English Bay off West Vancouver, BC, on April 28 and 29, 1998 and off Vancouver Island on May 9 and 10, 1998, and immediately transported to the West Vancouver Laboratory holding facilities.

In addition to the predatory fish that were held in the experimental apparatus (WCS and raceways), a stock of ten kelp greenling and one quillback rockfish were housed outdoors in a 2500-L tank, supplied with air equilibrated seawater (salinity  $28.2 \pm 0.8 \%$ , temperature  $10.7 \pm 1.0$  °C, dissolved oxygen  $100 \pm 2\%$  of air saturation, TGP  $100.6 \pm 0.5\%$ , pH  $7.8 \pm 0.1$ ), under a natural photoperiod. An air stone connected to an electric air pump (Elite 800, Rolf C. Hagen Inc., Montreal, Que) provided additional aeration (a precautionary measure implemented to protect the fish in the event of a water flow disruption). To exclude avian predators, the tank was covered with 4-cm diameter plastic (vexar) mesh which was overlain with a woven black polyethylene sheet to provide shade and minimize visual disturbance.

Within days of acclimation to captivity, the predators were consistently feeding on juvenile fish, as has been documented for these species in nature (Rosenthal et al. 1988; Mace 1983; Murie 1995; Nemeth 1997a, b). The predators adjusted well to the laboratory environment with no deaths or escapements occurring throughout the holding or experimental periods. These fish were acclimated to the outdoor laboratory conditions for a period of at least four weeks prior to experimentation. All the species proved to be efficient in the pursuit and capture of juvenile chum salmon thereby confirming their suitability as predators for use in the challenge tests. The predators were fed a maintenance ration of juvenile chum salmon at 1.8% wet body weight d<sup>-1</sup> for kelp greenling and sculpins, and 1.1% wet body weight d<sup>-1</sup> for rockfish.

All of the predators that were held at the West Vancouver Laboratory survived capture, holding, and experimentation before being returned to the wild on September 29

1998. Loading densities of these fish were adjusted in the holding tanks and in the experimental apparatus to ensure that the acclimation conditions contributed to the maintenance of their health.

#### VOLUNTARY VS FORCED EXPOSURE OF JUVENILE CHUM SALMON TO ELEVATED TEMPERATURE AND DGS

While the position of a school of chum salmon with respect to warm surface waters was previously investigated in both the laboratory and the field (Birtwell et al. 1998, 2001; Korstrom et al. 1998), information on their avoidance or preference behavior to these waters, and hence exposure of individual fish to the ambient conditions was lacking. Accordingly it was not possible to identify the time that a fish spent in the variety of conditions within the experimental water column, and only inferences were made of the potential consequences to chum salmon of exposure to warm seawater.

Our initial approach was to examine the susceptibility of juvenile chum salmon to predation by simultaneously exposing two groups of fish to the combined stressors of elevated temperature and dissolved gas supersaturation (DGS), and then challenging them to survive predation by piscivorous fish. We endeavored to determine if the volitional movement by chum salmon into seawater at temperatures at or above the 50% avoidance threshold (as determined in the laboratory), combined with DGS to levels as high as 130% TGP (a level that exceeded those recorded in Port Moody Arm; Birtwell et al. 1998), affected their susceptibility to predation. We used the WCS in which to conduct these preliminary experiments. Two groups of 20 chum salmon were placed into identical rigid mesh cages held in the WCS aquarium. One cage was orientated horizontally in warm seawater with DGS, and the other was orientated vertically such that its' uppermost portion was immersed in warm seawater with DGS, its' lowermost portion in seawater at ambient temperature and 100% TGP, and between there was a narrow thermocline. We anticipated that the fish given the opportunity to choose exposure to the warm waters might be less stressed than those fish without such a choice, and that this potential difference in exposure (and stress) could result in differential predation by the rockfish predators in the WCS. However, the juvenile salmon within the verticallyorientated cage which could choose exposure to the enclosed conditions and, potentially, avoid the warm seawater and DGS, chose instead, to occupy the warm waters rather than move deeper in the water column to more suitable and previously-selected temperatures (Brett 1952; Birtwell et al. 2001). The presence of the predators seemingly influenced the volitional movements of the chum salmon and overrode the behavioral thermoregulatory responses documented previously (Brett 1952; Birtwell et al. 2001). These fish chose, therefore, to move away from the predators thus becoming exposed to conditions similar to those of fish held without such a choice. This circumstance, of predators at depth and juvenile salmon biased to the water surface, has been documented in the wild and is, presumably, an inherent adaptive survival trait. Because of the potential for the similar exposure of each of the caged groups of juvenile chum salmon to the warm surface waters with DGS, and our corresponding inability to distinguish between exposures, we adopted a simpler experimental protocol. We chose not to compare the susceptibility to predation of a group of chum salmon given a choice of exposure to potentially stressful conditions with another group given a forced exposure. Instead we chose to compare the susceptibility to predation of a group of chum salmon given a predetermined exposure to warm seawater and DGS with a control group that was handled identically but not exposed to the DGS and warm seawater.

#### APPARATUS FOR EXPOSING JUVENILE CHUM SALMON TO ELEVATED TEMPERATURE AND DGS

The outdoor flow-through exposure apparatus comprised 12 cylindrical insulated polyethylene opaque tanks measuring 33.5 cm in diameter with water depth maintained at 43 cm by a central standpipe drain, enclosing a water volume of 36-L. The 12 exposure tanks were designated as follows: five tanks for control fish, five tanks for the exposure of chum to elevated temperature and supersaturated TGP (i.e. the treatment tanks), and two tanks for water quality monitoring (control and treatment waters).

The tanks were covered with sheets of styrene foam insulation in order to reduce heat loss, to provide cover for fish, and to reduce visual disturbance. Water was supplied to 1-L reservoirs mounted in the tank covers to facilitate mixing before it was discharged through polyethylene tubing to the tank bottom (displaced water exited via a central standpipe at the water surface).

Temperature in the designated water quality monitoring tanks was recorded every 15 min by data loggers (Onset Stowaway Tidbit<sup>™</sup> waterproof temperature logger, Onset Computer Corporation, Pocasset, MA; accuracy ± 0.2 °C, range -5 °C to 37 °C). Total DGS determinations ( $\Delta P$  and TGP %) were made using tensionometers (Model 300C, Alpha Designs Ltd., Victoria, BC; accuracy  $\pm 1 \text{ mm Hg}$ , range -200 to 700 mm Hg) and by applying the appropriate calculations provided by Colt (1984). To minimize erroneous readings caused by bubble accumulation on the probe membrane, the bubbles were dislodged by manually shaking the submerged probes prior to obtaining a reading. Water quality parameters were measured with the following meters: salinity (YSI Model 30 Hand-held salinity, conductivity and temperature meter, Yellow Springs Instruments Ltd., Yellow Springs, OH; accuracy  $\pm 0.1$  %, range 0 to 80 %), dissolved oxygen and temperature (Oxyguard Handy MKIII Portable DO meter, Point Four Systems Ltd., Port Moody, BC; accuracy  $\pm 2\%$  of air saturation, range 0 to 600%), and barometric pressure (Model 1215 Mercury Barometer, Sargent-Welch Co., Skokie, IL). The frequency of measurements in the control and exposure water quality monitoring tanks varied from one to four times daily, throughout each of the exposures of 12 to 48 hours duration.

#### **APPARATUS FOR DEEP-WATER PREDATOR CHALLENGE TESTS**

The Water Column Simulator (WCS) aquarium used for the deep-water predator challenge tests has been described by Birtwell and Kruzynski (1987) and, therefore, only a brief description will be given of the significant components. The WCS apparatus is housed in a self contained, sound-proofed, insulated, temperature and humidity controlled building to reduce visual and acoustic interference to the experimental fish. The WCS consists of a 4500-L acrylic aquarium ( $2.4 \times 2.4 \times 0.8 \text{ m}$ ) with three separate water circulation loops, which facilitate the formation of a horizontally flowing, homogeneous or vertically-stratified water column with controlled velocity (approximately 3-4 cm·s<sup>-1</sup>). Air-equilibrated seawater was continuously delivered to the flow-through aquarium at a rate of 60 L·min<sup>-1</sup> which corresponded to a 90% replacement time of approximately 3 h (Sprague 1969).

Overhead illumination to the WCS is provided by a metal halide light source (daylight spectrum) with seasonal photoperiod control. Photoperiod was adjusted bi-weekly during the experimental period to simulate natural seasonal changes using natural twilight tables provided by the National Research Council of Canada. Artificial dark : light cycles ranged from 4.5 h dark : 19.5 h light (including two 4.5-h periods of twilight representing dawn and dusk) in June, to 6.5 h dark : 17.5 h light in August. The aquarium was illuminated at night with near infra-red lighting by using an alternative light source shone through a red filter (50% transmission at 695 nm and at 722 nm with the peak at 705 nm). Fish behavior and movements were recorded for a 2-h period prior to the start of, and continued to the completion of, each predator challenge test ( $\leq$  90 min) by a high resolution camera (Panasonic WV-1850, 800 lines) with peak sensitivity in the near infra-red, coupled to a closed circuit black and white time-lapse video recorder (Panasonic AG-6750, 400 lines) and high resolution video monitor (Panasonic WV-5470, 850 lines). The aquarium, lighting systems, and camera were enclosed by vinyl curtains to occlude extraneous light and minimize visual disturbance of the fish.

To provide refuge and simulate natural habitat complexity, and to encourage an unconstrained interaction between predators and prey, clusters of large rocks were placed on the bottom of the aquarium together with artificial buoyant vegetation representing algal fronds.

#### APPARATUS FOR SHALLOW-WATER PREDATOR CHALLENGE TESTS

The shallow-water predator challenge test apparatus consisted of two insulated, 1600-L annular green fiberglass raceways (depth 41 cm, width 46 cm). These outdoor raceways were elevated 30 cm off the ground and situated adjacent to each other. Clear Plexiglas<sup>™</sup> (10-mm thickness) overlain with black polyethylene covers minimized external disturbances and algal growth. Ambient overhead illumination was provided, and light entered the raceways through two aluminum cylindrical apertures (25-cm diameter, 40-cm height) positioned through the covers on each of the straight sections of the raceway. Air-equilibrated seawater was supplied to the raceways, and an air stone connected to an electric air pump (Elite 800, Rolf C. Hagen Inc., Montreal, Que) provided additional aeration (a precautionary measure implemented to protect the fish in the event of a water flow disruption). Water was continuously delivered to the tanks at a rate of 25  $L \cdot \min^{-1}$  which corresponded to a 90% replacement time of approximately 3 h (Sprague 1969). A water current (approximately 4 cm·s<sup>-1</sup>) was created by the use of a perforated PVC pipe which spanned the width of the raceway channel at the apex of a curved section and which admitted jets of water into the tank from above the water surface thereby causing a movement of water around the raceway.

To provide refuge for the experimental fish, mimic natural surroundings and encourage interaction between predators and prey, the complexity of the habitat in each raceway was increased by the presence of clusters of large rocks.

A schematic diagram of the main components of the deep- and shallow-water predator challenge test apparatus is presented in Figure 1.

#### **GENERATION OF HEATED SEAWATER**

A two-stage boiler system heated the seawater for use in the experiments. The main components of the heating system included municipal and experimental water pumps, a titanium heat exchanger (Model Superchanger, Tranter Inc., Wichita Falls, TX), a primary electric hydronic boiler (Model Super Hot, Allied Engineering Company, North Vancouver, BC), and a secondary gas-fired hydronic boiler (Model Mini-Therm II, Teledyne Laars, Oakville, ON). Municipal water was heated by an electric element in the primary boiler and pumped in a continuously circulating loop through the titanium heat exchanger where heat was transferred from the municipal water to the experimental water by cross current exchange. The water temperature was monitored by a sensor in the reservoir. In situations where the first stage electric boiler was incapable of attaining the set point temperature, the second stage auxiliary gas-fired boiler was activated. Heating of water in a closed system results in dissolved gas supersaturation (DGS). The supersaturated (108.3  $\pm$  1.0% TGP), heated (20.7  $\pm$  0.7 °C) seawater was delivered to a 3500-L insulated, constant-head reservoir which provided a continuous flow of water at a constant temperature (i.e.,  $20.7 \pm 0.7$  °C) for the preliminary phase of each experiment involving the exposure of chum to elevated temperature and DGS. The heated water was circulated by pump between the reservoir and the electric boiler and heat exchanger.

# GENERATION OF DISSOLVED GAS SUPERSATURATION IN HEATED SEAWATER

Supersaturated seawater (TGP to 145%) was generated by exposing water to pressurized air within a 25 cm x 350 cm sealed and packed PVC column (Point Four

Systems Ltd., Port Moody, BC). The magnitude of the pressure and adjustment of water flow rates regulated the degree of DGS that occurred.

The control of the seawater level in the pressurized column was maintained through dynamic equilibrium. Seawater was supplied from the heated water reservoir by a magnetic drive pump ( $^{1}/_{3}$  horsepower, max. capacity 135 litres·min<sup>-1</sup>, maximum head 11.9 metres, Iwaki Co. Ltd., Tokyo, Japan) through a PVC ball valve (20 L·min<sup>-1</sup>) to enter the top of, and to be dispersed down the pressurized column through a bed of plastic packing material (Koch flexi-rings). The packing fractured the incoming water and provided a large surface area for the absorption of gas. The level of supersaturation of the seawater was controlled by adjusting the supply of compressed air to the column via a flow meter. If the gas addition rate was increased, the water level in the column would be lowered, exposing a greater depth of packing, thereby resulting in a higher absorption capacity of the column. If gas addition rate was reduced, the water level rose and the depth of exposed packing and absorption capacity were correspondingly reduced.

The level of water in the pressurized column fluctuated until a steady state position was reached commensurate with the gas addition rate. Water level was controlled automatically via a solenoid valve on the compressed air supply line, and an adjustable water level sensor attached to a "sight glass" on the side of the column. The water level sensor was moved to the desired height on the sight glass which corresponded with the water level to be maintained. If the water level fell to the level of the sensor, the coupled solenoid valve shut off the flow of compressed air to the column. With no air entering the column, the water level rose again until the level of the sensor was reached, at which time the supply of compressed air was renewed resulting in the water level dropping again. As the solenoid valve cycled on and off, the water level was maintained between the upper and lower limits of the sensor's "dead band" (approximately 6 mm).

Water at the desired temperature and supersaturated TGP level exited through the bottom of the pressurized column by gravity, and from there was distributed to the chum salmon exposure apparatus. Due to the complexity of changing the dissolved gas ratios of the heated seawater, the dissolved oxygen concentrations were not altered but were documented, and the % nitrogen (+ argon) saturation values and  $O_2/N_2$  ratios were calculated according to the equations of Nebeker et al. (1979) and Colt (1984). TGP levels of 115%, 120% and 130% in 20.7 °C water exiting the column corresponded with calculated mean  $O_2/N_2$  ratios of  $0.91 \pm 0.04$ ,  $0.91 \pm 0.04$ , and  $0.92 \pm 0.03$ , respectively. These  $O_2/N_2$  ratios did not differ appreciably from those prevailing in the summer of 1997 in the surface waters ( $\leq 1.5$  m) of Port Moody Arm. For example, approximately 70 m from the BGS thermal discharge at an average temperature of  $19.2 \pm 1.6$  °C, and TGP of  $110 \pm 2.9\%$ , the  $O_2/N_2$  ratio was  $0.94 \pm 0.13$ . Table 14 provides additional information on the  $O_2/N_2$  ratio for specific sites and depths in Port Moody Arm.

# GENERATION OF AIR-EQUILIBRATED SEAWATER AT AMBIENT TEMPERATURE

Seawater at ambient temperature was admitted to the experimental apparatus as required. This water was air-equilibrated by passing the water through 2.4 m high packed (Koch flexi-rings) columns, which resulted in dissolved gas levels of approximately 100% of air saturation. A mean  $O_2/N_2$  ratio of  $0.96 \pm 0.05$  was calculated for this water.

#### **RESISTANCE OF JUVENILE CHUM SALMON TO ELEVATED TEMPERATURE AND DGS**

Four preliminary bioassays were conducted between May 28, 1998 (fish weight  $1.57 \pm 0.22$  g, fork length  $58 \pm 2$  mm) and June 11, 1998 (fish weight  $1.92 \pm 0.23$  g, fork length  $61 \pm 2$  mm). Each of these preliminary tests used one group of 30 juvenile chum salmon at each of four levels of TGP, to determine their time to death. Fish were transferred from the stock tank to the exposure apparatus and acclimated for 30 min to air-equilibrated ambient seawater ( $10.0 \pm 0.5$  °C) before exposure to heated water ( $20.3 \pm 0.4$  °C) with 120%, 125%, 130%, or 140% TGP. For each of these preliminary tests, observations of behavior, location in the tank, progression of external signs of gas bubble trauma (GBT), and lethal exposure times were recorded until termination of the bioassay at approximately 85% mortality. The fish were considered dead when opercular movement ceased.

# EXPOSURE OF JUVENILE CHUM SALMON TO ELEVATED TEMPERATURE AND DGS

#### Pre-exposure

The experimental period was from June 12, 1998 (when fish weight was  $2.07 \pm 0.18$  g, and fork length  $63 \pm 2$  mm) to September 28, 1998 (fish weight  $15.17 \pm 2.80$  g; fork length  $120 \pm 5$  mm), excluding the time between August 11 to 23, when an epizootic of *Vibrio* sp. required treatment with antibiotics. Based on the results of the preliminary resistance bioassays, the duration for sublethal exposure of various groups of chum to elevated temperature and levels of TGP were decided on. At 20.7 °C, no mortality was expected after exposure to 115% TGP for 48 h, and  $\leq 10\%$  mortality was expected after exposure to 120% TGP for 24 h and 130% TGP for 12 h. Accordingly, these three combinations of exposure duration and TGP level were included in each treatment series.

Preceding each experiment, groups of approximately 100 fish were randomly selected and removed from the stock tank, and transferred to a 77-L holding tank which was continuously supplied with air-equilibrated seawater (salinity  $28.4 \pm 0.8$  %, temperature  $12.8 \pm 1.0$  °C, dissolved oxygen  $96 \pm 6\%$  air saturation, pH 7.5  $\pm 0.2$ ). Groups of 5-10 fish were anaesthetized in 13-L containers of seawater (pH  $7.1 \pm 0.2$ ) and 45 mg·L<sup>-1</sup> tricaine methanesulfonate (MS-222<sup>™</sup>, Syndel Laboratories Ltd., Vancouver, BC) which provided a sufficient concentration to cause equilibrium loss in approximately 2 min. Individuals of approximately equal size were then size-selected for use in the subsequent predator challenge experiments, to ensure that the length and weight of control and treated groups were not significantly different upon introduction to the predators. The weight (g) and fork length (mm) of each anaesthetized fish was recorded. Only those fish which appeared to be in good health were selected. In alternate tests at the same treatment level, either the control or treated groups of chum salmon were marked for later identification by removing the adipose fin, or, sham clipped and handled as if being marked. The fish were then allowed to regain equilibrium before transfer to the appropriate designated tanks in the exposure apparatus for acclimation. The marking procedure resulted in 1% mortality occurring during the fin clipping process and no mortality during the subsequent recovery and acclimation period. Initially, 30 fish were placed into each exposure tank, however over the experimental period fish growth necessitated a decrease to 20 fish and eventually 15 fish per tank in order to maintain a loading density <10 kg·m<sup>-3</sup> (Sprague 1973). Fish density was maintained at  $5.0 \pm 2.4$  kg·m<sup>-3</sup> with the same number of fish placed in both control and treatment tanks.

The fish were not fed, and were left undisturbed in the exposure tanks to allow recovery from handling stress for 18 h - 30 h prior to experimentation. During acclimation, the exposure tanks were supplied with ambient temperature (11.5 ± 0.6 °C), air-equilibrated seawater (salinity 28.5 ± 0.8 ‰; dissolved oxygen 97 ± 5% air saturation; TGP 100.6 ± 0.9%; pH 7.8 ± 0.1). All fish holding criteria were within standards established by Sprague (1973). Water flow during acclimation was delivered to the tanks at 5 L·min<sup>-1</sup>, which provided a 99% replacement of the tank water volume within 36 min and provided a flow-loading of  $\leq 0.08 \text{ kg}\cdot\text{L}^{-1}\cdot\text{min}^{-1}$ .

#### Exposure

Water in each of the treatment tanks was slowly heated for 10 min by delivering a mixture of 2.5 L·min<sup>-1</sup> air-equilibrated ambient (11.5 °C) seawater and 2.5 L·min<sup>-1</sup> TGP-supersaturated heated (20.7 °C) seawater. Subsequently, the flow of air-equilibrated water was terminated and the flow of the heated dissolved gas supersaturated water increased to 5 L·min<sup>-1</sup> for the duration of the exposure period. This water flow regime facilitated a rapid transition (0.25 °C·min<sup>-1</sup>) to treatment water temperature and DGS level and ensured minimal heat loss from the test tanks. The exposure period for treatment fish began when the temperature and TGP level had stabilized in the tanks (within approximately 30 min following the start of the transitional period for equilibration). Control fish were exposed to air-equilibrated seawater at 11.5 °C over the same time period. The fish were not fed during the exposure period.

Observations of behavior, progression of external signs of GBT, and lethal exposure times in the treatment tanks were made and recorded. Control groups were simultaneously observed for any signs of GBT and to assess any incidental mortality due to handling stress or exposure to the laboratory conditions. Both control and treatment fish used the entire water depth provided by the 43-cm tanks. Therefore, it was assumed that the exposures were conducted at sea level and this negated the requirement to correct TGP measurements for the effects of hydrostatic pressure compensation by the fish. If the treatment fish had sought depth to compensate for the effects of hydrostatic pressure a correction factor of 11 mm Hg per 15 cm of tank depth (approximately 4.3% TGP) would have been required to accurately reflect the TGP levels to which the fish were exposed. Because the fish used the entire water column during the exposure period, we concluded that the chum salmon were exposed to the TGP values that were recorded.

#### Post-exposure

For purposes of clarity, and throughout this report, the treatments imposed on the juvenile chum salmon will be referred to as T+115, T+120, T+130, to indicate the exposure to 20.7 °C seawater and the respective exposure times of 48 h, 24 h, and 12 h to TGP levels of 115%, 120% and 130%.

Following the exposure period, the treatment fish were subjected to a gradual change in temperature and TGP to pre-exposure conditions. Transition to air-equilibrated and lower temperature conditions was accomplished by delivering a mixture of 2.5 L·min<sup>-1</sup> airequilibrated seawater at 11.5 °C, and 2.5 L·min<sup>-1</sup> heated dissolved gas supersaturated seawater (20.7 °C), to each of the test tanks for 10 min. Subsequently, the flow of heated supersaturated water was terminated and the flow of the air-equilibrated and cooler seawater increased to 5 L·min<sup>-1</sup> for the duration of the post-exposure period  $(37 \pm 7 \text{ min})$ . This water flow regime facilitated a rapid and uniform transition  $(0.18 \pm 0.03 \text{ °C} \cdot \text{min}^{-1})$  to control tank water temperatures and approximately 100% dissolved gas saturation levels. This protocol allowed the water in the treatment tanks to decrease to a temperature that was within  $1.2 \pm 0.5$  °C of the temperature of the water in the predator challenge test tanks and thereby avoided a cold shock to the fish. Control fish were exposed to air-equilibrated seawater at 11.5 °C over the same post-exposure period. The time interval between the end of exposure to elevated temperature and TGP and the introduction of the juvenile chum salmon into the predator challenge test tanks (including the post-exposure period) as prey was  $45 \pm 7$  min.

Fish were transferred to the predator challenge test tanks from the exposure tanks, and this brief handling was most probably stressful. However, transit time was kept to a minimum by locating the exposure apparatus proximal to the predator challenge test tanks. Handling procedures during transfer were standardized and were the same for control and treated groups of fish.

### ASSESSMENT OF GBT IN JUVENILE CHUM SALMON

Immediately prior to release into the predator challenge test tanks, ten fish were selected at random from one or a combination of the control and treatment exposure tanks to establish the prevalence and severity of signs of GBT. The fish were rapidly removed by dip net from the exposure tanks and euthanised in MS-222<sup>TM</sup> (200 mg·L<sup>-1</sup>) dissolved in air-equilibrated seawater from the exposure tanks. The fish were then transferred into vessels containing air-equilibrated seawater where they remained for 5-30 minutes until necropsied. Fish from the control and treatment tanks were examined concurrently, and fish in each group were examined consecutively. Each sample of ten fish required approximately 30 min to process.

The body of each fish necropsied was examined externally for any gross lesions or signs of GBT. Exopthalmia is associated with GBT and is caused by the accumulation of gas within the fatty tissues of the periorbital space, resulting in abnormal protrusion of the eye from the socket (Stroud and Nebeker 1976). Occurrence of exopthalmia was noted and the eyes were examined for opacities or other abnormalities.

Fish were then placed, left side up, on the stage plate of a dissecting microscope (Wild M-5 Stereo-microscope, 6 to 50x magnification, Wild Heerbrugg Ltd., Switzerland) equipped with a low voltage illuminator (6 v, 15 w, 2.5 amp, tungsten lamp) on an articulated arm of an adjustable attachment bracket, the intensity of which could be regulated by a 110-v transformer. Stroud and Nebeker (1976) found that in trout, gas accumulations in the inter-ray membranous tissue and within the venules adjacent to the cartilaginous rays (subcutaneous emphysema) of fins occurred more frequently in the caudal fin than in the other fins and Weitkamp (1976) found that emphysema appeared first in the caudal fin followed by the other fins. Therefore, only the caudal fin was examined in the present study, and the percentage of coverage of this fin by subcutaneous emphysema was estimated and recorded. Schiewe and Weber (1976) showed that gas emboli formed in the scale pockets of the trunk lateral line of fish exposed to dissolved gas supersaturated water. As a consequence, the ability of this sensory system to then respond to stimuli was either diminished or eliminated. Therefore, the lateral line was assessed to determine the incidence (frequency of occurrence) of signs of GBT and the extent of its' occlusion. On the left side of the fish, the percentage of the length of the lateral line that was occluded by gas emboli was estimated and recorded. The severity of these two signs of GBT was assigned using the following ranking system: 0 = no emboli or emphysema present; 1 = 1% - 5% covered; 2 = 6% - 25% covered; 3 = 26% - 50%covered; and 4 = >50% covered. Any additional observations regarding fish condition that may have been related to GBT, such as small petechial hemorrhages in the lateral line and caudal fin, were noted.

Lastly, the opercula on the left side of the fish was removed (treated fish only) and the first gill arch was excised with surgical scissors and placed on a glass slide. The gill arch

was then covered with a few drops of seawater and examined for intravascular gas emboli using the dissecting microscope at 50 x power. The presence or absence of gas emboli in the afferent lamella gill vessels or filaments was recorded.

Fish that died during the T+130 exposure were examined for signs of GBT only at the end of the 12 h exposure period in order to minimize disturbance to the surviving fish. Thus, all dead fish remained in the dissolved gas supersaturated water until necropsied, and there was a maximum elapsed time of 12 h between death and necropsy. Necropsy procedures were the same for the dead fish as for the treated and control fish which survived the exposure period.

The ability of fish exhibiting signs of GBT to recover following transfer to airequilibrated conditions has been reported (Dawley et al. 1976a; Weitkamp 1976). The potential recovery of fish from the signs of GBT over the 45-min post-exposure period during which the fish were returned to pre-exposure conditions, was investigated. Thirty fish were necropsied immediately following the T+130 exposure and the severity and prevalence of the above-mentioned signs of GBT were compared to those in 30 fish examined after the 45 min post-exposure period in air-equilibrated 11.5 °C seawater.

### SHALLOW-WATER PREDATOR CHALLENGE TESTS

During the initial shallow-water predator challenge tests, one sculpin and one kelp greenling were held in each of the two 1600 L, 0.41 m-deep raceways supplied with air equilibrated seawater (salinity  $28.3 \pm 0.9$  %; temperature  $10.9 \pm 1.0$  °C; dissolved oxygen  $89 \pm 5\%$  air saturation; TGP 99.1  $\pm 0.8\%$ ; pH 7.7  $\pm 0.1$ ). Additional numbers of kelp greenling were transferred from the stock tank to the raceways for inclusion in predator challenge tests as required, in order to maintain the predator consumption rate in relation to the prey growth rate. The predator biomass in each raceway was also adjusted for the 2-3 predation trials performed each week. A sufficient time interval between predation trials was determined for the recovery of a consistent predator appetite. Hunger was standardized by depriving predators of feed for a minimum of 48 h immediately preceding each test. The number of kelp greenling in each of the raceways was increased from one to eight over the experimental period. Newly-introduced greenling were acclimated to the raceway, and to the resident greenling and sculpin predator population, for at least 48 h prior to a test. No predator was included in a predator challenge test until it had demonstrated a repeated ability to capture and consume juvenile chum salmon.

All challenge tests were initiated in mid-morning. During the earlier part of the experimental period using the shallow-water apparatus, two predator-challenge tests were performed simultaneously in the raceways. However, as the prey grew, loading density restrictions in the 12 available exposure tanks did not allow sufficient numbers of prey to be exposed concurrently and thus only one raceway could be utilized for the predator challenge tests at these times.

At the end of the post-exposure period, 20 treated fish and 20 control fish were removed by dip-net from one or a combination of exposure tanks, and combined in a 13-L transfer container, holding  $11.7 \pm 0.7$  °C air-equilibrated seawater, which was covered with a dark lid. A hatch in the Plexiglas<sup>TM</sup> cover of the raceway was removed, and the transfer container was slowly lowered into the raceway and oriented into the current thereby permitting the fish to swim out. The raceway cover was then replaced. This method of release ensured an initial dispersal of the chum fry in the raceway, with little obvious disturbance of the predators.

Based on a prior assessment of consumption rates, predation was allowed to continue for 60 min. The 60-min criterion was chosen after a number of preliminary trials had demonstrated that this time interval was sufficient to allow the predators to consume approximately 50% of the chum salmon prey without becoming satiated. Tests in which up to 70% of prey were consumed were accepted for analysis. At the conclusion of each test the surviving chum salmon were removed within two to five minutes, counted, and assigned to the treated or control group by the presence of, or lack of, their adipose fin. At this time, measurements of water quality were made in each raceway.

### **DEEP-WATER PREDATOR CHALLENGE TESTS**

For the first six weeks of the predator challenge tests, five rockfish (quillback and copper) were held in the WCS aquarium which was supplied with air equilibrated salt water (salinity  $28.3 \pm 0.7$  ‰, temperature  $10.8 \pm 0.1$  °C, dissolved oxygen  $101 \pm 2\%$  air saturation, TGP 100.4 ± 0.9%). On July 24, 1998, the remaining rockfish (quillback) was transferred from the stock tank to the deep-water aquarium thus increasing the resident population to six rockfish. This action was taken to more evenly distribute the size range of predators in the tank, minimize inter- or intra-specific competition, and reduce the potential formation of dominance hierarchies.

As with the predator challenge tests using the raceways, those using the Water Column Simulator apparatus were also initiated in the mid-morning. Twenty treated fish and 20 control fish were removed by dip net from one or a combination of exposure tanks and combined into a white nylon mesh basket inside a 13-L container, holding  $11.7 \pm 0.7$  °C air-equilibrated seawater, and which was covered with a dark lid. The container was slowly lowered into the surface waters of the WCS and gently removed leaving the prey enclosed within the mesh basket. The prey were held in the mesh basket for 2 min before it was slowly tilted and removed, thereby permitting the fish access to the entire water column of the aquarium.

Predation was allowed to continue until the earlier of one of the two following events had occurred: approximately 50% of the prey were consumed; or 90 min had elapsed. The 90-min limit was chosen to restrict the potential for the chum salmon to recover from the effects of GBT while in the air-equilibrated waters of the aquarium as well as during

the preceding 45-min post-exposure period. At the conclusion of each test, the surviving prey were removed within three to five min, counted and assigned to the treated or control group by the presence of, or lack of, their adipose fin. At this time, measurements of water quality were made in the aquarium.

### **ROCKFISH PREDATION EFFICIENCY**

During the predator challenge tests performed with rockfish using the WCS, the predator-prey interactions were observed through slits in the light occlusion curtains surrounding the apparatus. An additional black curtain was positioned behind the observation slit to enclose the observer and prevent disturbance of the fish.

It was expected that differences in predator performance might reflect changes in the activities and vulnerability of the groups of treated and control juvenile chum salmon. Brown et al. (1985) inferred that if individuals from a certain treatment group were harder to capture, the predator would have a low capture success (i.e. a low number of captures per attacks) indicating that the prey from this group were responding more quickly (or naturally?) than those from those fish of the other treatment groups. An assumption for the analysis of predator efficiency was that all the rockfish predators were equally experienced and similar in their abilities to capture the prey.

The total number of attacks (successful and unsuccessful) by the rockfish on the chum salmon was recorded by observers during each of the predator challenge tests. An attack was scored when it was clear that a particular prey was being pursued or when predators burst into the center of a school. The number of successful prey captures was verified at the end of the test when the surviving fish were recovered. The number of unsuccessful attempts to capture prey was calculated by subtracting the number of successful captures from the total number of attacks that were initiated. To account for the variation in the number of predators used per test during the experimental period, predator efficiency data were expressed on a per-predator basis. The data were analyzed to determine if the ratio of successful attacks per unit effort varied among treatments.

# ATTACKS BY ROCKFISH ON SOLITARY AND SCHOOLED JUVENILE CHUM SALMON

Morgan and Godin (1985) showed that fish which separated from a school were preferentially attacked and more successfully captured compared to those within the school, and that straying from the school had an associated increased risk of mortality due to predation. Because exposure to elevated temperature and TGP may stress fish, and potentially disrupt schooling with consequent effects on predation success, video recordings of the deep water predator challenge tests were reviewed to determine if solitary fish were preferentially attacked compared to those in a school. The video recordings could not, however, discern whether the predation events were directed at treated or control fish.

For each test, and for all treatment levels, each attack initiated by a predator against a solitary or schooling fish was scored. The overall proportion of attacks by rockfish on prey in isolation or those positioned within the school was calculated for each treatment level. Subsequently, the data were analyzed to investigate whether any relationships existed between the proportion of attacks on solitary prey and the prior treatment conditions experienced by 50% of the prey population. The data were also examined to determine whether the schooling behavior of juvenile chum salmon provided an anti-predator benefit against rockfish predation, irrespective of treatment.

### **BEHAVIOR OF ROCKFISH AND JUVENILE CHUM SALMON**

The behavior of the rockfish predators before the introduction of the chum salmon prey and during the challenge test was examined by analysis of the videotape recordings. This analysis was carried out to discern any difference in the behavior of the fish among the experiments that would assist in explaining any differential selection of the prey among treatments. The description of the procedures used and the results of these observations are provided in more detail in Fink et al. (1999). Videotape recordings from each predator challenge test which included chum from each of the three treatment levels were analyzed to provide information on the position of predators and the school of chum salmon in the aquarium, the presence of solitary fish, and the size of the chum salmon school and its' variation over time, the behavioral repertoire of the predators during attacks on the chum salmon, and maximum escape speed ( $V_{max}$ ) of the salmon.

## Position of rockfish before and after release of juvenile chum salmon

The position and general behavior of rockfish predators prior to, and after, prey (i.e., juvenile chum salmon) introduction was determined by recording the position of individual fish on a two dimensional 20 x 20 grid which corresponded with the internal dimensions of the WCS aquarium. Using the videotape time signature, a series of 15 observations at one minute  $(\pm 1 \text{ s})$  intervals was made prior to the introduction of prey. All predators visible at the time of each observation were assigned a grid location (column and row score) to indicate their relative position within the aquarium.

Preliminary testing within the WCS aquarium demonstrated that the behavior of predators before and after the introduction of juvenile chum salmon prey was different. Accordingly, different procedures were used to reveal their position during these two periods. Following prey introduction, and continuing for the duration of the experiment, an expanding time sequence was used to obtain samples of the predators' position. Four determinations were made at 5 s, 10 s, 20 s, 40 s etc., increasing time intervals until the conclusion of the experiment. The timing of these observations was, therefore, biased

towards the time of maximum predation activity, and thereby facilitated the recording and description of predatory events and fish behavior at such times.

### Location and size of school of juvenile chum salmon and the number of solitary fish

The position of juvenile chum salmon within the WCS during these experiments was determined in a manner similar to that used for the rockfish predators. However, instead of observations of individual fish, the approximate center of the school of salmon was estimated and assigned a grid location comprised of a column and a row score. The two dimensional size of the school of chum salmon was determined by the number of individual grids occupied by the school. In addition, the number of visible solitary fish that were not associated with the main school was determined.

# Maximum swim speed (V<sub>max</sub>) of juvenile chum salmon

Prey escape speed was examined (in two dimensions) in each predator challenge test to determine whether a change occurred over the experimental period, and among treatments. Time (s) and distance traveled (cm) were used to calculate the maximum escape speed ( $V_{max}$ ) of chum salmon. A maximum of five individual escape paths from each videotape record of each test was examined. Between six and eight individual time measurements, and up to five repeated distance measurements were averaged to produce a determination of  $V_{max}$ . Three to five such data groupings were combined to provide an estimate of the chum salmon escape speed for each test.  $V_{max}$  was expressed in cm·sec<sup>-1</sup> and also standardized to body lengths·sec<sup>-1</sup>.

### STATISTICAL TREATMENT OF DATA

Test data for predation were analyzed in a manner similar to that of Mesa (1994) and Mesa and Warren (1997). A number of additional statistical tests were used for comparative purposes and also applied to other data sets.

Data were analyzed primarily with nonparametric, distribution-free, statistical tests, as these tests do not make *a priori* assumptions about the normality of the distribution of the data and therefore the validity of the test is not affected by the distribution of any of the variables (Devore 1991). Parametric statistical methods are based on calculated means and standard deviations, the parameters of the normal distribution, while by contrast non-parametric statistics make no assumption about the nature of the distribution and therefore are free of assumed parameters (Norman and Streiner 1994). If assumptions of normality and homogeneity of variance are made about a distribution, and parametric tests are used in the analysis, then the conclusions drawn from those tests may be inaccurate if that assumption is shown to be false (Devore 1991). The major disadvantage usually attributed to the distribution-free tests is their lower power relative to the analogous parametric test (Devore 1991). An advantage of distribution-free tests is that many of them rank the raw

scores and operate on those ranks, and therefore offer a test of differences in central tendency that are not affected by one, or a few, extreme scores ("outliers") (Norman and Streiner 1994). In the present study, and wherever possible, both a distribution-tied parametric test and its corresponding distribution-free non-parametric equivalent were used to analyze the same data set, in an attempt to verify and confirm results.

At the conclusion of each predator challenge test involving chum from a control group together with an equal number of chum from a single treatment group (i.e., T+115, T+120, and T+130), the number of fish that were not consumed were identified and categorized as either treated or control fish. For the analyses, the reciprocal of this (or the data representing the number of fish consumed) was used. Using these data, the number of treated and control fish expected to remain at the end of each predator challenge test, had predation been non-selective between treated and control groups of juvenile chum salmon, was calculated. The analysis tested whether the frequencies of exposed and control salmon consumed by predators were different from release frequencies (i.e. 50:50). Observed and expected frequencies of treated and control prey consumed for each trial within a treatment level were compared with a non-parametric chi-square ( $\gamma^2$ ) goodness-of-fit test which is a simple method used to analyze categorical frequency data (Devore 1991). All predator challenge test data for an experimental treatment level were subjected to a heterogeneity  $\chi^2$  analysis to determine if the individual tests were homogenous (similar in distribution) and to validate the pooling of results from replicate tests. As recommended by Mesa and Warren (1997), data were pooled from all tests within a treatment level and a  $\chi^2$  goodness-of-fit test was used on the pooled data to determine if predation was random (i.e., 50:50) on treated versus control fish. The level of significance was set at p < 0.10 because the  $\chi^2$  test has low statistical power when relatively small sample sizes are used (Fairbairn and Roff 1980).

The experimental design attempted to limit prey consumption in predator challenge tests to 50%. Tests in which more than 70% of the prey were consumed were excluded from the analysis: the percentage of fish eaten was required to be standardized to account for the change in prey availability during a test where there was a relative decrease in the abundance of the preferred group as the experiment progressed (Bams 1967; Coutant 1973; Mesa and Warren 1997). Mesa and Warren (1997) excluded from analysis those tests where less than 30% of the prey were eaten for the stated reason that individual tests with a low number of fish eaten do not provide data for a valid  $\chi^2$  test, since expected values under the null hypothesis would typically be less than 5 (which violates a standard assumption for this statistical test). The present analysis included individual trials where less than 30% of the prey were eaten, as the lowest expected value was 4.5, which was a modest violation of the assumption for this test.

Hurlbert (1984) and Heffner et al. (1996) extensively reviewed published ecological literature and found substantial evidence of "pseudoreplication." "Pseudoreplication" was

defined as the use of inferential statistics to test for treatment effects with data from experiments where either treatments are not replicated (though samples may be) or where experimental units are not statistically independent (Hurlbert 1984). "Sacrificial pseudoreplication" results when an experimental design involves true replication of treatments but where data for multiple replicates are pooled under the same treatment prior to statistical analysis, which confounds sources of variation within the data set such as the variance among samples within an experimental unit and the variance among experimental units (Hurlbert 1984). Hurlbert (1984) concluded that the  $\chi^2$  test is one of the most misapplied of all statistical procedures and that when it is used specifically to assess treatment effects in manipulative experiments it seems to be invariably misapplied. Therefore, in addition to analyzing the predator challenge test data as did Mesa and Warren (1997), and in recognition of the concerns of Hurlbert (1984), an additional analysis of difference scores for each trial within each TGP treatment level was performed to avoid the "pseudoreplication" problem. This analysis was different than the tests of significance for categorical frequency data using the  $\chi^2$  goodness of fit tests discussed above, and was applied as a means of corroborating the results of that analysis. For each replicate within a TGP treatment level, a difference score was generated by subtracting the number of control fish consumed from the number of test fish consumed. For the purpose of statistical analyses, all replicates within a TGP treatment level were assumed to be independent and each represented a single observation. The difference scores for each TGP treatment level were analyzed using both the parametric distribution-tied one-way analysis of variance (ANOVA) test, and its non-parametric analogue the distribution-free Kruskal-Wallis oneway analysis of variance test which is used for independent samples and ranked data. To compare results at each TGP treatment level, an ANOVA was conducted on the difference scores between treatment and control fish consumed in each trial. A difference in the number of individuals captured was considered to be the result of a difference in vulnerability between the two groups (treatment and control) which were simultaneously exposed to the predators. This comparison enabled the statistical evaluation of a change in the vulnerability which resulted from impairment due to exposure to elevated temperature and TGP. Analyses were also conducted comparing difference scores to zero for each TGP treatment level using both the parametric paired sample t-test and its non-parametric analogue the Wilcoxon Signed Rank test (Devore 1991). The Wilcoxon Signed Rank test is used to compare two related, dependent samples (matched pairs of repeated observations), while taking into account the size of the rank order differences within the pairs (Norman and Streiner 1994). The level of significance was set at p < 0.05.

Determinations of water quality variables taken during control and treatment group exposures of chum salmon were compared with both distribution-tied analysis of variance (ANOVA) and distribution-free (Kruskal-Wallis) statistical tests. Where significant results were found, pairwise comparisons were conducted using the Tukey's Honestly Significant Difference (HSD) method. The Tukey's HSD procedure involves post-hoc comparisons and further exploration of the data after a significant effect has been found, as opposed to planned comparisons which are hypotheses specified before the analysis commences (Norman and Streiner 1994). For this analysis, data were pooled from all measurements taken during all tests conducted at each TGP treatment and control level. The level of significance was set at p < 0.05.

Mean lengths and weights of control and treated fish used in each predator challenge test were compared using both the parametric two-sample t-test and its analogue the non-parametric Mann-Whitney U test which is used to compare independent samples and ranked data. The level of significance was set at p < 0.05.

Data on the incidence of signs of GBT in chum salmon were "categorical frequency data" and therefore a non-parametric Fisher Exact test was conducted to determine differences between each treatment and control fish. Fish in TGP treatment levels or control groups with a low incidence of signs of GBT did not provide data for a valid  $\chi^2$  test, as some of the expected cell frequencies in the 2 x 2 contingency tables were less than 5. A Fisher's exact test is normally used as an alternative to a  $\chi^2$  test when the expected frequency of any cell in a 2 x 2 contingency table is less than 5 (Norman and Streiner 1994). For this analysis, data were pooled from all tests conducted at each TGP treatment level. The level of significance was set at p < 0.05.

The proportion of rockfish predator attacks initiated on prey in isolation, or those positioned within the school, was calculated for each treatment level. For each replicate test within a treatment level, a difference score was generated by subtracting the number of attacks against schooling fish from the number of attacks against solitary fish. The data were analyzed with a non parametric Wilcoxon Signed Rank test to investigate whether any relationships existed in terms of the proportion of attacks on solitary prey, and the treatment conditions experienced by 50% of the potential prey group prior to the predator challenge tests. The level of significance was set at p < 0.05.

The data on the proportion of successful captures of prey by rockfish were analyzed to investigate whether any relationships existed in terms of the proportion of successful attacks and the treatment exposure conditions experienced by 50% of the potential prey group prior to the predator challenge tests. The individual tests within each experimental treatment level were conducted on different dates from those of other treatments representing the same experiment; and because the juvenile chum salmon were growing during this time, prey size was included as an independent variable to account for some of the variance between treatments. A parametric Analysis of Covariance (ANCOVA) was computed using both prey length and prey weight as covariates (Devore 1991). Where significant results were found, pairwise comparisons were conducted using the Tukey HSD method. No transformation of the data was necessary as probability plots indicated a normal distribution. The level of significance was set at p < 0.10 due to the relatively small sample size.

### RESULTS

# **RESISTANCE OF CHUM SALMON TO ELEVATED TEMPERATURE AND DGS**

The mortality of chum salmon in these preliminary resistance bioassays revealed that with an exposure to 20.3 °C seawater and either 140%, 130%, 125%, or 120% TGP, the times to 10% mortality were approximately 2 h, 12 h, 24 h, and 24 h, respectively, and for 50% mortality they were approximately 19 h, 34 h, 40 h, and 44 h.

Based on these results we chose to expose juvenile chum salmon to 20.7 °C seawater for 12 h at 130% TGP, 24 h to 120% TGP, and for 48 h to 115% TGP prior to their release with an equal number of control fish into the predator challenge apparatus.

As mentioned previously, the treatments imposed on the juvenile chum salmon will be referred to as T+115, T+120, T+130, to indicate the exposure to 20.7 °C seawater and the respective exposure times of 48 h, 24 h, and 12 h to TGP levels of 115%, 120% and 130%.

### EXPOSURE OF CHUM SALMON TO ELEVATED TEMPERATURE AND DGS

### Water quality

Table 1 shows the mean  $\pm$  SD values determined for the variables that were monitored during the exposure of the juvenile chum salmon to the warm seawater and levels of TGP.

For the variables  $\Delta P$ , TGP, N<sub>2</sub>, and dissolved oxygen, the values between treatments were significantly different from each other and the controls. Values for the  $0_2/N_2$  ratio and temperature for the treatment groups were significantly different from the control values but not from each other. The analysis of salinity data revealed a significant difference between the T+120 and the T+130 treatment group (but not between other treatments or control); however the difference in salinity recorded among treatments was small (see Table 1) and is not considered to be biologically meaningful.

### **Chum salmon survival**

The exposure conditions to which the juvenile chum salmon were subjected prior to the predator challenge tests and related experiments resulted in few mortalities, and the survival statistics are as follows:

99.95% (2224/2225)
98.9% (714/722)
97.9% (647/661)

### Signs of GBT

The incidence and percentage of fish showing signs of GBT, for each treatment and sign recorded, are shown in Table 2. As expected, the percentage of control fish exhibiting any of the signs of GBT recorded was very low. Only 0.2% of the control fish that were examined was observed to have gas emboli in the lateral line, and only 4.2% and 1.3% of the control fish had hemorrhage in the lateral line and caudal fin, respectively. However, with the exception of eye exopthalmia or bubbles in the gills, which were only found in fish that had died during exposure to T+130, the percentage of fish from each of the treatment groups which showed other signs of GBT was significantly greater than the respective percentage of control fish displaying the same signs (Table 2).

### <u>Gills</u>

None of the live fish that were examined from the treatment and control groups of chum salmon immediately before the predator challenge tests had gas emboli in the gills. However, all the fish that had died during the T+130 exposure had gas emboli in the gills.

### Eyes

Exopthalmia was at a low incidence and the number of fish showing any evidence of this GBT sign was significantly elevated in only the T+120 (1.3% of exposed fish) and T+130 treatment groups (1.3% of fish) relative to the control group (0% incidence), and there was no significant difference among the treatment groups.

### Severity of signs of GBT in lateral line and caudal fin

The results of an assessment of the severity of the signs of GBT in the lateral line and the caudal fin are presented in Table 3. Only in the dead fish and in those fish which survived exposure to T+130 was there more than a 5% occlusion of the lateral line. In the caudal fin the majority of fish that were examined (and with the exception of the dead fish) also had emboli in <5% of the fin. We chose, therefore, to assess the differences in emboli in the lateral line and the caudal fin between groups of experimental fish on the basis of the incidence of the sign of GBT rather than use a severity ranking because of the low frequency of occurrence of fish displaying >5% occlusion of the lateral line, or >5% coverage in the caudal fin.

### Lateral line

Any signs of emboli and hemorrhage in the lateral line were most frequently observed in the fish that had died during the exposure to T+130 (Table 2). Similarly, the

percentage of fish displaying these signs of GBT were also highest in survivors of this treatment group of chum salmon relative to all the others that had survived the exposure to warm seawater and elevated levels of TGP.

The percentage of fish showing signs of emboli in the lateral line was not significantly different between the T+115 (2.7%) and the T+120 (6.7%) treatments, but it was significantly different between all other comparisons (Table 2). For each of the groups of treated fish, significantly more fish showed signs of emboli in the lateral line than did the controls. The highest incidence of this sign of GBT (43.3%) was in the fish that had died during the T+130 exposure, but of those surviving this exposure 27.1% had emboli in the lateral line.

The percentage of fish showing signs of hemorrhage in the lateral line was highest in the T+130 treatment group (27.7%) and significantly different to the incidence in the T+115 (17.3%) and the control groups (4.2%) (Table 2). For each of the groups of treated fish, significantly more fish showed signs of hemorrhage in the lateral line than did the controls.

### Caudal fin

The percentage of fish showing signs of subcutaneous emphysema and hemorrhage in the caudal fin was determined for each treatment (Table 2). Emphysema was not found in the control group, whereas each of the treatment groups displayed an incidence of this GBT sign which ranged from 5.6% to 10.3% (Table 2). The highest percentage of fish showing signs of emphysema in the caudal fin was found in the group of fish that died during exposure to T+130, in which instance 65.6% of these fish showed signs of subcutaneous emphysema in the caudal fin. Unlike this finding, only 9% of the fish that survived exposure to T+130 showed any signs of this effect due to GBT (Table 2). There was no significant difference in the incidence of fish showing any signs of emphysema in the caudal fin, among the fish that survived exposure to each of the three treatment levels. The incidence was significantly different, however, between that for all surviving fish and those which had died during exposure to T+130.

The percentage of fish showing signs of hemorrhage in the caudal fin was greatest in the T+130 treatment group (15.2%) and similar to that in the T+115 group (15.0%). The incidence of fish showing this sign of GBT did not differ significantly among treatments, but it did differ significantly between each of the treatment groups and the control group (1.3%), as shown in Table 2.

### **Disappearance of signs of GBT**

This assessment was taken to provide an indication as to whether some overt recovery from the signs of GBT (and potentially from debilitation and stress) was possible in the

37-min period between the end of the prey exposure period and before the predator challenge test began.

There was no significant difference in the percentage of fish showing signs of GBT in the eyes, gills, lateral line, or caudal fin of juvenile chum salmon at the end of the exposure to T+130 and those percentages for the group of fish that were examined after exposure to the same conditions but following the 37-min period when temperature and TGP were decreased to ambient levels prior to the predation challenge test (Table 2).

### PREDATOR CHALLENGE EXPERIMENTS

# Kelp greenling and Pacific staghorn sculpin predation on juvenile chum salmon: shallow-water challenge tests

Five experiments were excluded from the data set because the number of prey consumed did not fulfill the requirements of the test and lay outside the maximum 70% consumption level. One experiment used fish at the start of the *Vibrio* sp. disease outbreak. In this test 80% of the treated fish (T+115) and 50% of the control group of chum salmon were consumed by the predators. Despite more of the treated fish than control fish being eaten, we could not discount the potential effect of the additional stress due to disease biasing the final result and hence we also excluded this result from the data set that was analyzed.

We endeavored to permit the predators in the shallow raceways the opportunity to feed over 60 min, and during this time we expected that they would consume approximately 50% of the prey (a deduction from preliminary consumption tests). Because we could not observe the feeding rate of the predators and because we did not wish to disturb them in order to determine the numbers of chum salmon consumed during the course of a test, there was no way of determining when the ideal test objective of 50% consumption of prey had been met.

The predators consumed an average of 5.4% of their wet body weight during each of the predator challenge tests (minimum 2.2%, maximum 9.7% of their wet body weight).

# Size of prey used in predator challenge experiments

The selection of juvenile chum salmon of similar size for use in the experiments was undertaken to minimize the potential bias in results that could be caused by the predators selecting smaller fish. To examine the success of this selection process, and to examine the potential for a biased predator prey selection, the length and weight data for the respective treated and control groups of fish used in each of the predator challenge tests was subjected to statistical analysis. These data are shown in Table 4. Of the 45 paired (treated and control fish) groups of chum salmon used in these predation tests, the statistical analysis revealed that only in one test was the length and weight of the treated fish significantly different than those of the control fish (fork length of control vs treated fish 95 ± 3 mm vs 93 ± 4 mm and weight  $7.5 \pm 0.85$  g vs  $7.1 \pm 0.8$  g). There was, however, no evidence of a size selective bias for the consumption of smaller fish and 10 of 20 treated fish and 11 of 20 control fish remained at the conclusion of this test. In the other challenge test wherein there was revealed a statistically significant difference in size of the two prey groups, only the weight (and not the length) of the treated fish was significantly greater than that of the control fish (2.3  $\Box$  0.33 g vs 2.0 ± 0.29 g respectively) but again, there was no similar bias in the consumption of prey (more of the heavier treated fish than the control fish had been consumed) which inferred a sizeselective discrimination by the predators.

### Consumption of treated and control prey

The data for the percentage and numbers of juvenile chum salmon consumed by the predatory fish during the 15 tests carried out on either T+115, T+120, or T+130 treatments and associated controls in the shallow water raceways are presented in Tables 5 to 7. Figures 2 to 4 show, respectively, the relative consumption of treated and control chum salmon prey for each shallow-water predator challenge test.

### Control and T+115 treatment

The results of the individual predator challenge tests comparing the consumption of control and treated groups of fish are shown in Figure 2, and presented with the results of statistical analyses in Table 5. More of the treated fish were consumed in 8 of the 15 predation challenge tests. The pooled consumption data (Table 5) show that slightly more of treatment than control fish were consumed (expressed as a percentage of all the fish that were consumed in a test), and that the differences for each test and for the pooled data (0.3%) were not significant. In addition, the difference scores (i.e. the difference between the number of chum salmon from the treated group compared to the number from the control group that were consumed) were compared to zero using a paired t-test and Mann - Whitney U test. These tests revealed no significant difference in the consumption of treated vs control chum salmon and accordingly corroborated the results of the  $\chi^2$  test.

### Control and T+120 treatment

The results of the individual predator challenge tests comparing the consumption of control and T+120 treated groups of chum salmon are shown in Figure 3, and presented in Table 6 together with the results of statistical analyses of the data. More treatment fish than control fish were consumed in 11 of the 15 tests, however there was no significant differences in consumption of the two prey groups for an individual test. The analysis of the pooled data revealed that the difference (i.e., 9.8% more treated than control fish

consumed, of the total consumed) was significant (p < 0.1). Table 6 also displays the difference score between the treatment and control fish that were consumed. The results of the paired t-test and the Mann - Whitney test revealed a significant difference (p < 0.05) thus corroborating the results of the  $\chi^2$  test for the pooled data.

### Control and T+130 treatment

The results of the individual predator challenge tests comparing the consumption of control and treated groups of prey are shown in Figure 4, and also presented in Table 7 together with the results of statistical analyses of the data. More treatment fish than control fish were consumed in 10 of the 15 tests, however the difference in consumption of the two prey groups for each test was not significant. The analysis of the pooled data revealed that the difference (10.7%) was significant (p < 0.1). Table 7 also displays the difference score between the treatment and control fish that were consumed. The results of the paired t-test and the Mann - Whitney test revealed a significant difference (p < 0.05) thus corroborating the results of the  $\chi^2$  test for the pooled data.

# <u>Differences between number of treated vs control juvenile chum</u> salmon consumed among treatments

The relative differences in the percentage of treatment and control chum salmon that were consumed in all of the tests are shown in Figure 5. In 29 of 45 tests (i.e., 64.4% of all shallow-water predator challenge tests performed), more treatment fish than control fish were consumed. The results of statistical analyses revealed that there was no significant difference in the consumption of treated vs control chum salmon between the treatment levels.

# ROCKFISH PREDATION ON JUVENILE CHUM SALMON: DEEP WATER CHALLENGE TESTS

A total of 19 tests were carried out in the deep water WCS aquarium, but one of them was excluded from the data set because of the potential for disease to have affected the results. In this test, 70% of the treated chum salmon group and 30% of the control group were consumed.

# Rockfish behavior prior to introduction of the juvenile chum salmon

Before each introduction of juvenile chum salmon into the deep water aquarium, the quillback rockfish and the majority of the copper rockfish positioned themselves around the mid tank area or just shallower, and were generally oriented into the current and biased towards the upstream left side of the aquarium (Figure 6). The activity level of individuals was generally low. Occasionally, one or more rockfish would make rapid "exploratory" movements which would temporarily alter the position of the other fish.

After several minutes, the fish gradually assumed positions similar to those previously occupied. Because of the presence of cover at the bottom of the tank (rocks and artificial plants), not all rockfish were visible prior to the introduction of the chum salmon.

### Introduction of chum salmon

Upon release of the juvenile chum salmon from the mesh basket into the aquarium, they dispersed from the release area in a combined outward and downward direction. In early experiments, and before the rockfish had become habituated to the routine of prey introduction, the movement of the prey was usually unaffected by the movement of the predators for the first 5 - 10 s and during that time, the chum salmon would quickly occupy locations within the enclosed water body of the aquarium. In later experiments the dispersal of prey throughout the aquarium was interrupted by the attacks of the rockfish which caused a rapid dispersal of individuals from the release area. This dispersal was followed by a return of the chum salmon to the surface waters to form a school.

Within 30 - 75 s following introduction into the WCS, and after the initial dispersal of the chum salmon, a school was formed which gradually diminished in size as the rockfish attacks continued, and as the number of surviving prey diminished. In every experiment this school developed quickly and was very close to the water surface. Thereafter, and except for the time immediately following an attack, individual fish rarely ventured to depths greater than 24 cm.

### School location and size

The location of the main school of chum salmon within the WCS during the predator challenges is shown in Figure 7. The location and movement of the school throughout each test was similar. When the school was established, the chum salmon quickly moved towards the surface of the aquarium; a characteristic trait of juvenile salmon to occupy shallow near shore surface waters (Birtwell and Kruzynski 1987; Birtwell et al. 1998, 2001). The school remained within 24 cm of the surface for the duration of each experiment. When rockfish approached, the school of chum salmon moved away from them. If this approach was made near the middle of the school, it would occasionally split for a few seconds before re-forming. More often, however, separated individuals would move quickly past the predator at the water surface, rather than swim under the rockfish in order to rejoin the school.

Regardless of the prior treatment, changes in school size with time, after introduction of the juvenile chum salmon into the aquarium, followed a predictable pattern that was repeated, with minor variation, in each of the predator challenge tests. Figure 8 depicts the change in the size of the school of chum salmon during the predator challenge tests on treated and control groups of juvenile chum salmon. The size of this school typically decreased over time until 100 - 200 s from the start of the test, after which it usually stabilized.

## Rockfish behavior after introduction of chum salmon

Following prey introduction, rockfish movement increased. Both their position in the tank and their overall behavior were substantially different from that immediately prior to the introduction of the prey. The positions of the rockfish following the release of the chum salmon into the WCS are shown in Figure 9. The change in location and dispersion of the rockfish contrasts with that shown in Figure 6, before the introduction of the prey. Rockfish were able to see the juvenile chum salmon thus there was no requirement on the part of the predators to search for the prey except perhaps for those brief intervals when startled prey ventured into the artificial plant habitat in their effort to escape. Even at those times, the rockfish continued to pursue their prey through the plants.

Several characteristic attack sequences were observed during the predation periods and all, typically, involved ram feeding, or what we considered to be a combination of ram and suction feeding. Norton (1991) states that "in ram feeding the predator engulfs the prey via rapid acceleration of the whole body, often assisted by protrusion of the ascending process of the premaxilla. In suction feeding negative pressure generated by rapid expansion of the buccal cavity draws a jet of water into the mouth; this jet of water carries the prey into the buccal cavity (Lauder 1983)." These attack strategies were characterized as:

a) Slow stalk from distance followed by a smoothly accelerating attack. This was one of the more common attack strategies but it was, however, characterized by a high abandon frequency due, seemingly, to an unsatisfactory predator-prey alignment prior to the attack. The rockfish appeared to focus on a particular group or individual, orientated to face the prey, and then gradually accelerated towards it with only minor directional corrections.

b) *Instantaneous close range attack*. This attack appeared to be triggered by a minimum prey distance, and was characterized by both rapidly moving prey and/or another rapidly pursuing predator.

c) *Slow stalk to proximity with prey followed by an explosive attack.* This attack was commonly used against solitary prey, especially those momentarily stationary by the aquarium walls, or more particularly in the corner of the WCS aquarium. Prey against the walls of the aquarium often appeared to hesitate about which direction in which to escape, and this hesitation was often fatal.

d) *Close range attack (ambush) from cover*. Several rockfish (often the same individuals) appeared to use the shadow zone at the upper rear of the tank to approach the school of chum salmon, and then burst into its center from very close range.

The attack strategies used by the rockfish changed over time and within experiments. Immediately following the release of the prey and before they were, seemingly, aware of the potential danger, rockfish would slowly approach the salmon and then attack. As the chum salmon rapidly dispersed under predator attack pressure, the rockfish would chase rapidly-moving solitary prey throughout the aquarium and continue to do so until it was captured, or until it had joined the surface orientated school. This school was more resilient to disruption and less easily dispersed than the loose association of chum salmon that was present at the time of release, and predator attack sequences were seen to change as a consequence. At this time, the rockfish concentrated their attacks on solitary prey and also attacked the center of the school from cover. In the former case, a single chum salmon would often attract the attention of several predators at the same time.

The behavior of rockfish following an attack invariably followed one of two characteristic patterns. If the attack was unsuccessful in capturing a salmon, but the prey were still in close proximity, the rockfish would often initiate a second attack. If the prey were either captured or startled out of close range, the rockfish would swim to the bottom of the aquarium to either consume the captured prey or begin a new attack sequence.

### Attacks on solitary and schooled chum salmon

Analysis of videotape recordings revealed the presence of solitary chum salmon prey during the course of the predation experiments on groups of control and treated fish. Their presence was consistent among tests and treatments with the greatest number of solitary fish occurring at the start of the predator challenge experiment, and irrespective of the level of treatment (Figure 10).

The number of attacks that the rockfish made in relation to the behavior of the chum salmon prey was examined for each experimental treatment (admixed treatment and control fish) over the duration of the test, and also with the first five minutes of data eliminated. The latter analysis was carried out to assess the nature of the predatory attacks and to determine if it varied over time in relation to the decreasing number of solitary fish from the time of release, and the corresponding association with a school. Table 8 presents the data on the attacks which the predators made on solitary and schooled chum salmon for each of the three treatments (including their control groups), and for the data set which combined all treatment levels.

In each treatment group and for the combined data, and with and without the first five minutes of data, there were more attacks on solitary fish than on those in a school. However, statistical analysis revealed that there was a significant difference between the number of attacks on solitary vs schooled prey for each treatment level (61% vs 39%, 66% vs 34%, and 61% vs 39% of attacks on solitary fish vs schooled fish for the respective T+115, T+120 and T+130 treated fish admixed with control fish) only when the data from the entire test (i.e. not excluding the first 5 min) were used. These

differences were not significant between the three treatment levels. When the first five minutes of data were removed from the data set and the same analysis undertaken, no significant difference was determined between the number of attacks on solitary versus schooled chum salmon.

### Attack success

The data for the percent of successful attacks were analyzed to reveal differences in the capture success among treatments. While it was not possible to ascertain whether a treated fish or a control fish was attacked and/or captured because both groups were exposed to the predators simultaneously, we speculated that there may be differential predation success if the treated fish, because of potential debilitation, were not only captured more frequently but also more efficiently.

The success of the rockfish in capturing the chum salmon in each of the three experimental series (i.e. T+115, T+120, or T+130) is revealed in Table 9. ANCOVA determined that there was a significant difference (p < 0.1) between the percentage of successful attacks on the T+115 and control fish (17.1% per rockfish predator) and those on the T+120 and control fish (31.8% per rockfish predator). The same comparison of data for the T+120 and T+130 groups and their respective control fish did not reveal a significant difference.

The data presented in Table 9 also show that not only were there more predator attacks (14.6 per predator) on the population of chum salmon comprised of control fish and the T+115 groups, than on the other experimental groups (10.3 and 10.7 attacks per predator), but also that the efficiency of capture was similarly different.

The efficiency (expressed as a ratio of the number of successful captures to the number of attacks) with which the rockfish captured the chum salmon prey was 1:5.8, 1:3.1 and 1:3.3, in which the treated fish had been exposed to T+115, T+120, or T+130, respectively.

Thus, even though fewer fish were captured by the rockfish in the T+115 vs the T+120 and T+130 treatment groups, the predators still preferentially attacked solitary fish, had to attack more numerously, had a relatively lower number of successful attacks, and hence a reduced prey capture efficiency relative to predator attacks on the other treatment groups. The data infer that less energy was required to capture fish from the T+120 and the T+130 control and treatment groups (fewer number of attacks and greater success of capture) relative to T+115 groups. Because more treated fish were consumed relative to control fish, from the admixed populations (T+120 and T+130), there is the inference that the treated fish were preferentially selected.

### Maximum prey escape speed (V<sub>max</sub>)

Accompanying the increase in the size of the juvenile salmon during the course of these experiments was a natural and progressive increase in swim speed as the fish grew, which might have influenced the success of the rockfish in capturing the juvenile chum salmon. Maximum escape (burst) speed ( $V_{max}$ ) was used as a proxy for examining the ability of the chum juveniles to survive a predator attack. The overall increasing change in ( $V_{max}$ ) as a function of time of experimentation, and hence growth of the fish, is presented in Figure 11. During the 2-month period from June 24 to September 22,  $V_{max}$  was found to increase from 150 to 210 cm·sec<sup>-1</sup> (but relative to body length, the speed decreased from 23 to 17.6 body lengths·sec<sup>-1</sup>). The average standardized  $V_{max}$  was, however, very similar among the respective experiments (19.2, 19.4, and 19.9 body lengths·sec<sup>-1</sup> for experiments in which control fish were mixed with an equal number of fish that had been exposed to T+115, T+120, or T+130, respectively).

#### Size of prey used in predator challenge tests

The length and weight of the groups of chum salmon used in the predator challenge tests performed using the WCS apparatus are shown in Table 10.

Of the 18 paired (treated and control) groups of chum salmon used in these predator challenge tests, statistical analysis revealed that only in one test was the weight (but not the length) of the treated group  $(7.0 \pm 0.7 \text{ g})$  of fish significantly different than that of the control fish ( $6.6 \pm 0.85 \text{ g}$ ). However, there was no evidence of a size selective bias in the consumption of the lighter control prey, in that for these two groups, 45% (9 of 20) of the treated fish and only 30% (6 of 20) of the control fish were eaten.

### Consumption of treated and control prey

The data for the percentage and numbers of juvenile chum salmon consumed by the rockfish during six experiments in the deep-water (WCS) aquarium are presented in Tables 11 to 13 in relation to the treatment the chum salmon received prior to the predator challenge test. Figures 12, 13, and 14 show, respectively, the relative consumption of treated and control fish for each predator challenge test carried out with fish that had been given either T+115, T+120, or T+130 treatments.

### Control and T+115 treatment

The results of the individual predator challenge tests comparing the consumption of control and T+115 treated groups of fish are shown in Figure 12 and presented together with the results of statistical analyses in Table 11. More of the treated fish were consumed in four of the six predator challenge tests. The pooled consumption data (Table 11) show that, although more treatment than control fish were consumed overall

for these six tests, the differences between the pooled data for the treatment groups versus the control groups (6.5%) were not significant. In addition, the difference scores revealed no significant difference in the consumption of treated vs control chum salmon and corroborate the results of the  $\chi^2$  test for the pooled data. Thus, there was no significant difference in the consumption by the rockfish of T+115 treated versus control chum salmon.

### Control and T+120 treatment

The results of the individual predator challenge tests comparing the consumption of control and T+120 treated groups of fish are shown in Figure 13, and presented together with the results of statistical analyses in Table 12. More treatment fish than control fish were consumed in each of the six tests, however there was no significant difference in the consumption of the two fish groups for any test. The analysis of the pooled data revealed that the difference (22.8%) was significant (p < 0.05). Table 12 also reveals the significant difference (p < 0.05) in the difference score between the consumption of treated and control fish thus corroborating the results of the  $\chi^2$  test for the pooled data. Thus there was a significant difference in the consumption of T+120 treated vs control chum salmon by the rockfish.

### Control and T+130 treatment

The results of the individual predator challenge tests comparing the consumption of control and T+130 treated groups of fish are shown in Figure 14 and these data, together with the results of statistical analyses are provided in Table 13. More treatment fish than control fish were consumed in three of the six tests, and in each instance there was no significant difference in the consumption of the two fish groups for each test. The analysis of the pooled data also revealed that, overall, the difference in the consumption of treated versus control prey (11.3%) was not significant. Table 13 also reveals that the difference scores for the treatment and control fish that were consumed were not significantly different (p < 0.05), thus corroborating the results of the  $\chi^2$  test for the pooled data. Thus there was no significant difference in the consumption of treated vs control chum salmon by the rockfish, for this (T+130) treatment.

## <u>Differences between number of treated vs control juvenile chum</u> salmon consumed among treatments

The relative differences in the percentage of treatment and control chum salmon that were consumed (as a percentage of the total number of prey that were eaten) in all of the tests in the deep water aquarium are shown in Figure 15, for each of the three treatments and their respective control groups. In 13 of the 18 tests, more treatment fish were consumed than were control fish. The results of statistical analyses revealed that there was no significant difference between the treatment levels in the consumption of treated versus control chum salmon.

### DISCUSSION

### **GENERAL COMMENTS**

The environment in which fish live is dynamic and fraught with challenges that, if met, will facilitate their survival. While the maintenance of fish health and performance are critical to the meeting of such challenges, the inherent behavior of fish is intimately associated with these factors and their survival. Innate behavior has ensured the survival of individual fish, and hence populations over time, and is, presumably, adaptive. However, in the face of anthropogenic change to the aquatic environment and the associated creation of stressful conditions for fish, behaviors that previously have ensured their survival may become maladaptive and detrimental (Birtwell and Kruzynski 1989; Schreck et al. 1997). Not only do fish have to detect anthropogenic change of consequence to their well being, but subsequent actions must be balanced in the face of other ambient stimuli, and it is the adaptive response to the latter or the lack of detection of stressful factors that may compromise their survival and well being. Examinations of the behavioral responses of fish provide data relevant to an assessment of such factors. This opinion is reinforced by Schreck et al. (1997) who state that behavioral measures may be readily interpreted within an ecological context, thereby increasing the efficacy of extrapolating laboratory results to the field. However, it is the integration of behavioral responses by fish to the multiple cues in their dynamic environment that requires resolution, if a meaningful understanding of their adaptive capacity and response(s) to various environmental conditions is to be attained.

It is clear that dissolved gas supersaturation (DGS) and temperature extremes (or abrupt changes thereof) have the potential to stress fish and accordingly alter those behaviors which are essential to survival. Numerous studies have revealed the lethal effects of DGS (refer to Weitkamp and Katz 1980; Fidler and Miller 1997) and temperature (e.g. Brett 1952) on fish, but studies which attempt to identify, quantify, and provide the link between sublethal exposure to these variables, and other stressors, on fish survival and well being are relatively few despite their high relevance to understanding the ecological consequences of exposure to combined stressors. Research on the effects of temperature, in concert with other environmental stressors, has shown how some salmonid behavioral traits that are presumably adaptive may jeopardize health and survival in environments that are impacted directly or indirectly by anthropogenic activities (Coutant 1987; Birtwell and Kruzynski 1989; Birtwell et al. 1997; Korstrom et al. 1997; Jain et al. 1998).

Behavioral responses of fish to DGS have been exemplified through studies on swim bladder over-inflation and the fishes' subsequent avoidance through movement to depth (e.g. Shrimpton et al 1989; 1990a, b). However, not all fish species or life cycle stages may respond similarly, thus complicating the determination of potential ecological impacts of DGS (Fidler and Miller 1997, Fidler et al. 1999). The volitional response of some fish to move to deeper water to compensate for DGS has been examined in the laboratory and in experiments in the field. For example, Dawley et al. (1976a, b) exposed both chinook salmon and steelhead trout to DGS in deep tanks. In the deep (2.5-m) tanks, both species of fish tended to school and occupy waters at depth (around 1 m). The depth of the tank provided the necessary hydrostatic pressure to negate the effects of high (>120%) DGS and thereby permit survival of the salmonids if the fish chose to reside at or below the compensation depth. That deaths of individuals occurred with both fish species held in the deep tanks showed that some fish were unable (or unwilling) to totally compensate for DGS >120%. Dawley et al. (1976b) stated that "less hydrostatic compensation was derived due to depth disposition than expected when the mean depths of the fish groups are considered. Thus individual fish apparently moved substantially from the observed mean depth of the test lot." Dawley et al. (1976b) assumed that the fish were "moving randomly within their observed distribution." This result, of a temporary habitat shift to avoid aquatic conditions perceived as stressful, and subsequent mortality of some individuals despite the opportunity to survive at depth, is not unique. It has also been demonstrated for juvenile salmon that encountered toxic concentrations of pulp mill effluent in surface waters (McGreer and Vigers 1983; Birtwell and Kruzynski 1989).

Past observations that fish show signs of gas bubble trauma (GBT) in locations where water depth is available to provide the necessary hydrostatic pressure to compensate for DGS infer that the behavioral repertoires of the fish are such that individuals become exposed to the potentially stressful conditions (B. Antcliffe, Fisheries and Oceans Canada, Vancouver, BC; personal communication). It is apparent that the factors that provide a stimulus for the occupation of habitats in the wild need to be addressed in the course of laboratory experiments that seek to provide insights into the consequences of the volitional exposure of fish to stressors.

Our earlier experiments which examined the volitional behavior of chum salmon in the thermally-stratified WCS implied that chum salmon would behaviorally thermoregulate and in doing so seek temperatures that are optimal for metabolism (refer to Birtwell et al. 2001). However, as determined through experiments in Port Moody Arm (Birtwell et al. 1998), at times, the majority (>50%) of the experimental chum salmon occupied waters in which temperatures exceeded the laboratory-derived 50% avoidance threshold

(20.2 °C). This, we considered, was associated with the response to the more numerous cues in the wild versus those in the laboratory setting. The response of juvenile chum salmon during our initial experiments in which we had hoped to compare any selective

predation that occurred between chum salmon held, versus those that chose to enter, warm seawater with different levels of DGS revealed a movement of the chum salmon away from the predators and into the warmer and shallower DGS waters. Presumably this avoidance response under "natural" circumstances, is adaptive and in association with other cues results in these juvenile salmon occupying the uppermost part of the water column. Thus, it appears from laboratory studies and those in the field that juvenile chum salmon will strive to use surface waters despite the potential for adverse effects, from such variables such as elevated temperature and dissolved gases, which could occur with prolonged residency in these waters.

# EXPOSURE OF JUVENILE CHUM SALMON TO ELEVATED TEMPERATURE AND DGS

The exposure of chum salmon to warm seawater and different levels of DGS during the initial resistance bioassays revealed a more rapid mortality of fish exposed to the higher (i.e., 130% and 140%) levels of TGP. Because elevated levels of TGP (typically >100% to <115%) were recorded in the surface waters of Port Moody Arm together with temperatures >20 °C in the summer of 1997, we decided to expose juvenile salmon to a range of conditions that encompassed, and exceeded, the single maximum TGP value (125%) recorded (Birtwell et al. 1998).

Previous research has shown that elevated levels of TGP, if sufficiently high, can be lethal to juvenile salmon; and furthermore that when combined with elevated temperature, the mortality rate is exacerbated (Jensen et al. 1986; Fidler et al. 1999). The time to the onset of mortality in populations of fish decreases with increasing temperature while the rate of mortality increases with increasing temperature (Nebeker et al. 1979). According to Fidler et al. (1999), these relationships appear to be exponential, with very short times to initiation of mortality and very high rates of mortality at 20 °C and above. The reasons for this are considered to be associated with the increased metabolic activity of the fish and the need for the increased transport of oxygen to the tissues which, concurrently increases the transport of dissolved nitrogen thereby increasing the rate at which dissolved gases accumulate in body compartments. As temperatures rise above 20 °C and approach the lethal limits for salmonids (Brett 1952), other factors compound the response of fish to GBT. For example, losses in oxygen availability in the water and insufficient oxygen transport capability by the fish due to high temperatures compound the stress caused by GBT (Fidler et al. 1999).

Although populations of juvenile chum salmon are known to reside in estuarine, nearshore areas for days to weeks (Mason 1974; Levy et al. 1979; Healey 1980, 1982; Simentsad and Salo 1980) and subsequently occupy near-shore marine habitats (Healey 1979, 1980, 1982) before moving to the Pacific ocean, we do not know about the potential exposure of individual fish to the surface water conditions in Port Moody Arm. While an examination of the volitional dispersal of chum salmon held within cages in Port Moody Arm in 1997 revealed a distribution that was integrated over one day within 1-m depth intervals, it was not possible to determine the exact position of individual fish during this period, and hence their specific exposure to the ambient conditions (Birtwell et al. 1998). Notwithstanding this lack of knowledge, it is clear that these fish may reside in waters that are potentially harmful with prolonged occupation, and that avoidance of adverse surface water conditions may be a transient phenomenon (Birtwell and Kruzynski 1989).

Shrimpton et al. (1989, 1990a, b) determined that juvenile salmonids (<55 mm) may move deeper in the water column to compensate for DGS. These results were obtained in the laboratory and in the absence of natural cues. It is possible that the response of similarly sized fish to DGS in the wild may be mediated by, if not controlled by, such factors as the presence and availability of food and predators. To this extent it is probable that the juvenile salmon in Port Moody Arm may choose to occupy the shallow surface waters and maximize their chances of survival by balancing the risks of predation against the need to feed and grow using adaptive behaviors (Metcalfe et al. 1987; Magnhagen 1988; Lima and Dill 1990; Godin 1990) that have contributed to their survival over time. We deduced that it was probably not unreasonable for juvenile chum salmon to reside in the surface waters of Port Moody Arm for days to weeks (Levy et al. 1979), and that because of adaptive behaviors they may encounter sub-optimal surface water conditions during that time. Therefore, we chose to expose the juvenile salmon to a maximum of 48 h to T+115, 24 h to T+120, and 12 h to T+130, exposure times which ensured the survival of the majority of the exposed fish. We considered that the duration of exposure to the lowest TGP level was conservative considering the likelihood of chum salmon residence in Port Moody Arm being longer than 48 h. Similarly, temperatures greater than the laboratory-derived 50% avoidance threshold of 20.2 °C were often recorded in the shallow surface waters in the early summer and, accordingly, under a worst-case scenario, we could have chosen a much longer duration of exposure, and a temperature above 20.7 °C (refer to Birtwell et al. 1998). Because we chose a relatively short duration of exposure to the test conditions, it is possible that the more tolerant and resistant fraction of the test populations would not have been adversely affected by exposure to the TGP levels used. However, we considered that the low mortality of chum salmon incurred during the exposure periods indicated that at least some fraction of the population of these fish was probably stressed by the imposed conditions of elevated temperatures and DGS. Mesa and Warren (1997) considered that fish displaying a significant severity of signs of GBT would be more susceptible to predation irrespective of the previous history of exposure which resulted in these conditions. Thus, although we chose levels of TGP that were either exceptional (120%) or exceeded (130%) recorded values in the waters of Port Moody Arm, the duration of exposure to these levels in 20.7 °C seawater would be expected to produce equivalent signs of GBT in chum salmon that were exposed to lower levels of TGP but for periods longer than the 48 h we used in the T+115 treatment, and furthermore that the signs would occur more rapidly in higher temperature waters (Fidler et al. 1999; Jensen et al. 1986). Although the fish that were

exposed to the various levels of TGP had the choice to remain at depth in the 43-cm deep test apparatus and thereby obtain the benefit of increased hydrostatic pressure (and an equivalent reduction of about 4.3% TGP), the fish moved throughout the water column. Therefore, the nominal experimental exposure level was most likely very close to that which the fish experienced over the exposure periods. The results of statistical analyses revealed that there was a significant difference in the levels of TGP among the experiments but that temperatures were not significantly different. Salinity was significantly lower for the T+120 treatment group but the difference (0.1 - 0.4%) was not considered to be biologically meaningful.

The 20.7 °C temperature to which the chum salmon were exposed was not expected to produce mortality, based on the thermal resistance results of Brett (1952). Juvenile chum salmon acclimated to 10 °C and suddenly immersed in water at 23 °C or 24 °C had respective median times to death of approximately 6.8 d, and 15 h (Brett 1952). Acclimation to higher temperatures affects the level of the upper lethal temperature to salmon, but eventually an acclimation level is reached beyond which increased resistance to higher temperature for juvenile chum salmon in fresh water the ultimate upper lethal temperature for juvenile chum salmon was determined to be 23.8 °C (Brett 1952). Because the temperature used in the exposure experiments reported herein was at a level at which about 70% of the test fish were found to avoid increasing temperatures in laboratory experiments (Birtwell et al. 2001) we anticipated that this temperature alone may be sufficient to induce stress in the fish.

Thus, the periods of exposure that were chosen, and the levels of the variables, were such that mortalities were very low, and within acceptable limits even for controls that may be used in some comparative bioassay experiments (Environment Canada 1998). Accordingly, we consider that the treatments we administered to the juvenile chum salmon were at a sublethal level for the majority of these fish.

### Signs of GBT

An examination of subsamples of each group of treated fish, after exposure but prior to the predator challenge experiments, revealed signs of GBT that others have deduced to be related to debilitation and an enhanced vulnerability to predation (Mesa and Warren 1997). We did not, of course, sample the fish that were used in the predator challenge tests and therefore we can only infer the likelihood of a similar frequency of occurrence of the signs of GBT that occurred in the samples we examined, to that in the fish used in the predator challenge tests.

All of the fish that had died during exposure to T+130 had emboli in the gills, and significant numbers of these fish had emboli in the lateral line and sub-cutaneous emphysema in the caudal fin. No fish that survived exposure to T+130 had emboli in the gills. This result contrasts with that of Mesa and Warren (1997) who found, surprisingly,

surviving juvenile chinook salmon with emboli in the gills after 3.5 h exposure to 130% TGP in waters at  $15.2 \pm 0.1$  °C: a result that indicates a severe effect. The presence of emboli in the gills is usually followed by death within minutes to hours (Fidler 1998a, b). In Mesa and Warrens' (1997) experiments, exposure of chinook salmon to 130% TGP for 3.5 h resulted in the mortality of about 20% of the fish whereas the exposure of chum salmon to T+130 for 12 h only resulted in 10.2% mortality. This difference in results between studies most probably reflects the differing responses among species of salmonids to TGP and temperature, rather than an osmotic effect due to the use of seawater versus fresh water (the chum salmon used in the present studies were fully acclimated to seawater, and this species of salmon adjusts rapidly and progressively during the transition from fresh water to seawater (McInerny 1964; Mason 1974)).

It has been shown that the susceptibility of fish to GBT is highly dependent on species. age class or size, and temperature (Weitkamp and Katz 1980; Fidler and Miller 1997). Nebeker et al. (1979) found that a sea level TGP of approximately 126% had no effect on eggs or newly-hatched fry of steelhead trout (Oncorhynchus mykiss). However, at a DGS level of about 117% TGP, this resistance continued until the fish were about 16 days old, at which time bubbles began to form in the mouth, gill cavity, and yolk sac (Nebeker et al. 1979). The accumulation of bubbles in larval fish compromises swimming and feeding ability and the fish may eventually become trapped at the water surface as a result of excess buoyancy (Jensen 1980). Juvenile and adult fish appear to be more susceptible to GBT than newly-hatched fry or fingerling salmonids, with lethal signs first appearing at 110% - 118% TGP (Stroud and Nebeker 1976, Weitkamp and Katz 1980, Gray et al. 1985, Fidler 1988). In these fish, sub-dermal emphysema of the mouth lining accompanied by blockage of gill water flow by extracorporeal interlamellar bubbles may be the cause of death in laboratory environments (Fidler 1988). At TGP levels of 115% - 118%, bubble growth in the cardiovascular system can lead to death in just a few hours (Dawley and Ebel 1975, Dawley et al. 1976b, Stroud and Nebeker 1976, Fidler 1988, Fidler 1998a).

In general, the signs of GBT in juvenile chum salmon immediately following the prescribed treatments occurred most frequently in those fish exposed to T+130. Except for those fish which had died during the exposure period, the overall percentage of test fish showing any signs of GBT, for those groups exposed to this or the other (i.e., T+120 and T+115) treatments, was generally low, indicating a less severe effect of exposure on the fish than that recorded by Mesa and Warren (1997) for chinook salmon after 3.5-h exposure to 130% TGP. There was no difference in the percentage of fish showing signs of hemorrhage or sub-cutaneous emphysema in the caudal fin among the treatment groups, but all such incidences were significantly different than the controls. However, the incidence of fish showing any signs of emboli in the lateral line was similar between the T+115 and T+120 treatment groups (2.7% and 6.7%, respectively) and different from the T+130 (27.1%). Contrasting with this result, Mesa and Warren (1997) reported that

after an 8-h exposure to 120% TGP or a 3.5-h exposure to 130% TGP, all chinook salmon had emboli in the lateral line.

Based on the assumption that the prevalence and severity of the observed signs of GBT are related to debilitation of the affected fish (Coutant and Genoway 1968; Mesa and Warren 1997) and that this debilitation may lead to a compromise of sensory and motor abilities thereby potentially influencing vulnerability to predation, one may expect that there would be a greater consumption of all treatment groups of fish relative to control fish. Furthermore, it would be expected that there would be a difference in the consumption of chum salmon among the treatments that was reflective of any differences in the health and fitness of individuals. Overall there was a low incidence of the signs of GBT in all treated fish and there was no obvious abnormal behavior displayed by the chum salmon prior to their use in the predator challenge tests.

### PREDATOR CHALLENGE TESTS

### **Behavior of prey and predators**

The appetite of the predators for the chum salmon prey placed into the deep-water WCS aquarium or the shallow-water annular raceways was maintained throughout the experiments, although it was not always consistent. The need to balance the consumption of the predators in the challenge tests with the requirements for a significant proportion of the experimental population of test fish to be eaten (Bams 1967; Coutant 1973) required that an appropriate appetite of the predators had to be maintained through hunger, but without compromising the health and performance of the predators. Brett (1971a) reports the strong linkage between hunger and appetite in fish and it is apparent, therefore, that the predatory fish assemblages in the respective apparatus were held in a state of hunger that was sufficient to maintain appetite without adversely affecting their performance in capturing and consuming prey.

The response of the chum salmon prey upon release from the mesh enclosure into the WCS was consistent among experiments (Fink et al. 1999). Although the rockfish quickly became habituated to the introduction of the prey within the mesh holding basket and generally occupied a position in close proximity, it is not certain that the chum salmon recognized the potential danger of predation. Olla and Davis (1989) determined that the exposure of coho salmon (*O. kisutch*) to lingcod (*Ophiodon elongatus*) enhanced their chances of survival through learning. Ginetz and Larkin (1976) considered that predator-experienced sockeye had enhanced chances of survival from rainbow trout predators in contrast to predator-naive individuals. Healey and Reinhardt (1995) demonstrated not only the increased vulnerability of predator-naive versus experienced juvenile chinook and coho salmon to adult rainbow trout, but also the differences in this vulnerability between species. We have no information that would indicate whether the chum salmon that were placed in the WCS had increased awareness of potential predation

through their brief holding in a mesh enclosure within the WCS, prior to release. However, the chum salmon quickly dispersed from the loose school that was present immediately upon release, especially in response to attacks from the predators.

The response of chum salmon to seek depth and cover when startled or frightened, has been observed and reported by Hoar (1958) and Beall (1972). The presence of artificial algal fronds and rocks on the bottom of the WCS provided minimal cover for the chum salmon, and those individual fish that did venture to depth were vigorously pursued by the rockfish (Fink et al. 1999). It appeared that the presence of the artificial physical habitat in the WCS was not at a level of complexity that would have significantly affected the predator success. Savino and Stein (1982) noted that the predation by largemouth bass (Micropterus salmoides) on bluegills was reduced in relation to the increase in the complexity of habitat (artificial plants), and that not only did the predators change attack strategies as a consequence, but that the prey also modified their behavior and reduced schooling in relation to the increasing complexity of the habitat. In experiments in the WCS, the continued attacks by the rockfish soon after the introduction of the prey resulted in the formation of a school of chum salmon at the water surface. This behavioral trait of juvenile chum to school in very shallow (<1 m) waters has been recorded to occur particularly during downstream migration (Hoar 1958) transition from fresh water to seawater (Hiyama et al. 1972; Mason 1974; Iwata 1980; Beak Consultants Ltd. 1981), and during their early sea life (McGreer and Vigers 1983; Birtwell and Kruzynski 1989; Birtwell et al. 1998).

It is apparent that the chum salmon quickly learned of the danger posed by the predators, although proximity to a predator did not always trigger a flight reaction, as has been reported for other predator-prey interactions (Ydenberg and Dill 1986). The response of the chum salmon to form a school was relatively rapid and no doubt is an adaptive survival trait (Neill and Cullen 1974). Hoar (1951, 1958) also noted this trait for chum salmon in fresh water and Mason (1974) and Iwata (1980) for chum salmon entering estuaries and marine waters. In the experiments in the WCS, the stimulus to school was most likely associated with the capture of con-specifics as well as pursuit by the predators. In addition, the presence of chemical substances released from chum salmon and predators could have elicited the fright response and contributed to the observed behaviors (Rehnberg and Schreck 1987; Brown and Smith 1997). The formation of a school is considered to be a strategy by which potential prey may obtain the benefit of a confusion effect on the predator (Magurran 1990), through "swamping" (Taylor 1976; Peterman and Gatto 1978), and increased vigilance (Magurran 1990). This benefit of the close association of potential prey has been referred to as the "selfish herd" (Hamilton 1971) and as such is considered to confer a survival advantage to those animals in the center of the group. However, although numerous predators will attack and capture solitary prey (Morgan and Godin 1985; Parrish 1989) or those at the periphery of a school (Krause 1993), the benefit of presence within the school is only gained if the predators consistently attack its periphery and isolated individuals. It is

apparent that the predators' attack strategies are generally varied and are modified in relation to the prey type and behavior (Krause 1993). In the present series of predator challenge tests within the deep-water WCS apparatus, the rockfish attacked both the schooled and solitary fish, but disproportionately so.

In all experiments within the WCS, the rockfish attacked solitary fish in preference to the schooled fish irrespective of the preceding treatment given to 50% of the prey population (Table 8). This predatory behavior resulted in a significant difference between attacks at individuals versus the school within the early part of each experiment (i.e., within the first 5 min), but not thereafter. Thus, although attacks continued to occur on solitary fish after this time, there was not the same bias towards such fish as occurred just after the prey were released into the WCS and a discrete school had formed. There was no significant difference among the three experimental treatment groups, and in each case the rockfish attacked solitary fish more so than those in a school. It is possible that this difference reflected not only the relative ease of capturing control and treated predatornaive prey upon their release into the WCS, but also the diminishing numbers of prey, their quickly-learned avoidance strategies, and the behavioral adjustment of the predators to use a variety of attack strategies that would be more beneficial to capturing the prey. It is well documented that the attack strategies of piscivorous fish are modified depending upon the prey that they attack (e.g. Norton 1991). Because of the diverse diet of rockfish, sculpins, and greenling (Hunter 1959; Patten 1971; Mace 1983; Rosenthal et al. 1988; Armstrong et al. 1995; Murie 1995), each of these predatory species would be expected to employ appropriate attack strategies that were commensurate with their efficient capture of prey. It is not surprising, therefore, that the rockfish in the WCS used a number of attack strategies that resulted in their successful capture of chum salmon (Fink et al. 1999).

The benefit of schooling by the juvenile chum salmon as a means whereby predation pressure from rockfish was reduced was probably less in this artificial environment, which favored the predator, than would occur in the less-confining natural estuarine and marine habitats. The typical attack strategy of the rockfish observed in these studies was to use "ram" feeding (Liem 1980) on the mobile chum salmon prey (most piscivorous fish use ram feeding as a preferred method to capture elusive prey (Nemeth 1997a, b). The predators consistently captured prey through speed of movement and the use of different attack strategies. While the presence of the chum salmon in the school obviously conferred some immunity from attack during the early phase of each predator challenge test, the rockfish attacked the middle of the school in addition to attacking solitary fish. Thus, the benefit of the school (selfish herd) was diminished as the rockfish adjusted their attack strategies. This type of behavior was also noted by Parrish (1989) in relation to the feeding of black sea bass (Centropristis striata) on Atlantic silversides (Menidia menidia), and it was deduced by these researchers that the survival benefit of schooling was diminished in relation to the modified and differing attack strategies of the predators (Magurran 1990). The school itself was the target of attacks and while

schooling fish commonly stray as individuals and then rejoin the school, thus increasing their vulnerability when straying (Morgan and Godin 1985), there are examples in the literature where individuals in the center of a school have suffered the greatest number of attacks (e.g. Parrish 1989). In our experiments, the chum salmon displayed behavioral schooling traits that have been observed in the wild. Predation upon them was biased towards solitary fish that initially had dispersed from the school, and later upon those prey that had either strayed from it or which had been dispersed through predator attacks into the school itself.

Because the shallow-water predator challenge tests using kelp greenling and Pacific staghorn sculpin were carried out using an apparatus that did not permit observation of either prey or predators, we cannot provide comments on the attack strategies of these predators or the evasive tactics of their prey.

### Differential consumption of juvenile chum salmon and related factors

The predator challenge experiments, as a whole, resulted in the consumption of more treated than control chum salmon. This result was irrespective of the apparatus used and the mix of predators within them. However, significant differences occurred in the consumption of treated vs control fish depending upon the conditions to which the chum salmon were exposed.

In the deep-water aquarium of the WCS, rockfish predation on chum salmon exposed to T+115 or T+130 was not significantly different from that on control fish, but it was significantly different between the T+115 and the T+130 treatment groups.

In the shallower annular raceways, there was again no significant difference between the consumption by kelp greenling and Pacific staghorn sculpin of treated fish and control fish in those experiments in which the treated fish had been subjected to T+115. However in those shallow-water experiments in which the prey had been exposed to T+120 or T+130, there was a significant difference and more of the treated fish were consumed than the control fish.

Thus the data indicate the increased vulnerability of chum salmon to predation by four species of marine fish after exposure to the combined effects of elevated temperature and TGP. Despite the experimental conditions favoring the predator we obtained information that revealed differential predation on treated and control groups of fish within a treatment. Factors that may have influenced these results are discussed below.

### **Exposure conditions**

Previous research had determined that prolonged exposure to elevated TGP could prove-detrimental to the health and survival of aquatic organisms depending on such variables as the TGP level, life stage and species exposed (refer to Fidler and Miller 1997). Before the present series of controlled-exposure studies, however, the specific effect of the combination of warm seawater and elevated levels of TGP on chum salmon had not previously been investigated. Because elevation of temperature and TGP may affect the health and survival of chum salmon depending upon the duration of exposure and the level of the variables, we attempted to expose chum salmon to conditions which encompassed or exceeded those that had been recorded in Port Moody Arm (Birtwell et al. 1998), during the time of chum salmon residence in the area. Research by Coutant (1973; Coutant et al. 1979) and Mesa and Warren (1997) has shown how the exposure of juvenile salmon to elevated temperature or elevated TGP, respectively, increased their susceptibility to predation.

The choice of the duration of the exposure with respect to the three levels of dissolved gas supersaturation (DGS) used in these experiments was based on the results of preliminary resistance experiments. It is apparent from the survival data following exposure of juvenile chum to warm seawater and DGS, and an examination of the fish prior to the predator challenge tests, that the fish exposed to T+130 were probably subjected to the most stressful of the three treatments. It is undoubtedly likely that the treatments we imposed upon the fish were stressful, but a precise determination of the level of that stress and its significance was not apparent from the survival information and an examination of the fish prior to their use in the predator challenge experiments. We deduced, however, that the three treatments resulted in different levels of expression of the signs of GBT and that this inferred a potential change in the health status of the fish relative to the control groups. That said, there were no overt changes in the behavior of the chum salmon such as aimless or erratic swimming, loss of equilibrium, quiescence, lethargy (Coutant and Genoway 1968; Kruzynski et al. 1994; Mesa and Warren 1997) that indicated debilitation of the treated or control groups of fish.

## Water quality

Water quality did not vary significantly among the experiments and, except for those variables that we wished to control and test (i.e. temperature and dissolved gases), these factors were discounted as variables that could explain the differential low mortality of salmon during the exposure period, and the subsequent differential consumption of treated vs control chum salmon among treatments. All the juvenile chum salmon used in the study reported herein had been held in seawater for many weeks before being used in experiments and, therefore, were not expected to have suffered any osmotic stress which may have influenced the results we obtained. Rapid changes in salinity have been shown to induce stress (Handeland et al. 1996) and alter anti-predator behavior, making the fish more susceptible to attacks by predators (Järvi 1989a, b). The reaction distance of non-salt water adapted individuals, and the latter schooled more frequently. The net result of these changes in "anti-predator" behaviors due to osmotic stress was a reduced chance of escape from predators (Järvi 1989a, b; Handeland et al. 1996).

The  $0_2$ : N<sub>2</sub> ratios in the treatment groups were not significantly different from each other and were similar to those calculated for the waters of Port Moody Arm, close to the thermal discharge location in 1997. Data for the  $0_2$ : N<sub>2</sub> ratios calculated from information gathered in Port Moody Arm in 1997 are presented in Table 14. It is evident from these data that levels of N<sub>2</sub> frequently exceeded 100% saturation and contributed to the recorded elevated levels of TGP, especially proximal to the BGS thermal discharge point (sampling site 2, 70 m east of the discharge and within the thermal plume). Elevated levels of TGP in the waters closest to the surface often reflected the supersaturated levels of dissolved oxygen which was more evident further from the thermal discharge location, but at depth TGP levels were frequently elevated while dissolved oxygen levels were depressed, hence the waters were supersaturated with N<sub>2</sub>, the source of which was most probably the BGS thermal discharge (refer to Table 14). Changing ratios of dissolved oxygen to nitrogen under supersaturated conditions have been shown to affect the survival of fish. For example, Rucker (1976) reported a decrease in the lethal effect at 119% TGP when the oxygen and nitrogen partial pressure ratios were changed from 159%:109% to 173%:105%. Rucker (1976) also notes the protective effect of elevated pO2 on survival of coho salmon, and in hyperoxic environments (such as Port Moody Arm during daytime in late spring and early summer refer to Table 14), fish are more protected from the effects of TGP (Fidler and Miller 1997).

### Prey marking and abnormal behavior

The marking of individual chum salmon, by removal of the adipose fin, was alternated between the control and treatment fish to avoid the potential for the preferential selection of marked fish by the predators. Although the removal of a fin has been shown not to interfere with the swimming capabilities of goldfish (Radcliffe 1950), the creation of prey with specific abnormal characteristics has led in some studies to a reduction in survival through selective predation. Predators tend to attack the most vulnerable or obvious prey (Coutant et al. 1979). For example, it has been documented that depending on the strategies employed, some predators will selectively prey upon marked fish (Baker and Modde 1977; Coutant et al. 1979; Fresh et al. 1980), in addition to consuming those with modified or abnormal behaviors (Kruzynski and Birtwell 1994; Kruzynski et al. 1994), reduced performance capabilities (Bams 1967; Beall 1972), or those fish which had died (Gadomski and Hall-Griswold 1992; Petersen et al. 1994). Hargreaves and LeBrasseur (1985) reported, however, that coho salmon did not feed on dead pink or chum salmon prey. Based on the results of studies involving the predation on fin-clipped juvenile pink salmon by adult coho salmon, Hargreaves (1988) commented that although the predators preferred the smaller prey, fin clipping did not substantially affect the prey size selectivity.

### Predator appetite

The size of the chum salmon prey increased during the experimental period, and despite this there continued to be significant consumption of them by the predators throughout all studies. There was, however, an indication that the appetite of the predators was reduced more by the consumption of larger prey than by their consumption of smaller prey, because of the extended duration of some of the tests in the deep-water aquarium towards the end of the experimental period when the prey were largest. Brett (1971a) states that in general, larger prey take longer to process into flesh than an equivalent biomass of smaller prey, but one would expect that the satiation of the predators could occur quicker if they chose to consume the larger versus the smaller prey over the course of the experimental period. Brett (1971a) reported, however, that size itself does not affect satiation time but does influence food intake. Furthermore, the "handling time" of the prey by the predator increases as the fish approach satiation (Werner 1974). Thus it is possible that in those of our experiments in which the predators required a protracted period to consume the prey, that some of the factors just mentioned were acting together. We chose to adjust the number of predators in the shallow-water predator challenge tests rather than reduce the number of fish comprising the population subjected to predation pressure, and in this way we attempted, and succeeded in sustaining similar predation pressure among the tests. This manipulation was also considered to be more ecologically meaningful, and also revealed the potential for the consumption of a wide size range of juvenile chum salmon during their early marine life.

### Prey size

To minimize the potential for the predators to select prey of a particular size, both the treated and control groups of fish to be used in each predator challenge test were graded to be of approximately equal size. The results of statistical analyses on the groups of treated and control fish revealed that in all but three of the 63 tests there was no significant difference in size. In the three tests in which size was significantly different, this difference was not related to a size-biased consumption of treated or control fish. Norton (1991) determined that prey size had little effect on the capture success of cottids on shrimps over a limited range of prey size; however, a relationship between the size of the predator and the size of its prey has been recorded in other studies (Juanes 1994).

The chum salmon prey did not attain a length that exceeded the maximum length which may have resulted in their immunity from attack and ingestion by the predators. In general, piscivorous fish tend to most frequently attack prey that are 20% - 30% of their length, and that the maximum prey length is typically about 50% that of the predator's length (Juanes 1994).

The maximum mean length of chum salmon used in these predator challenge tests was

12 cm, representing 34%, 41%, and 39% of the mean length of the kelp greenling, rockfish, and Pacific staghorn sculpins, respectively. The minimum length of the prey was 6.3 cm, which represented 18%, 20%, and 21% of the mean length of the kelp greenling, rockfish, and Pacific staghorn sculpins, respectively. In studies on the predation of chum salmon by coho salmon in Masset Inlet, BC, Hargreaves and LeBrasseur (1985) determined that the maximum ingestible prey size for coho salmon was 40% to 50% of their length.

There is a great deal of information in the literature that indicates that smaller fish are often more vulnerable to predation than larger fish, but optimum foraging theory would predict that the fish would consume prey from which they derive the maximum energetic benefit. Clearly, the issue is very complex for there are examples where predators have selected larger prey over smaller ones (e.g., Main 1985; Marcotte and Browman 1986) and vice versa (e.g. Parker 1971; Hargreaves 1988), or the results have been equivocal (e.g., Coutant 1973; Ruggerone and Rogers 1983; Fresh and Schroder 1987).

Patten (1977) states that smaller prey tend to be selected by sculpins in the wild and that the maximum size of prey taken in the laboratory is greater than that in the field. Mace (1983) reports that the growth of juvenile chum salmon in the wild was such that it reduced predation pressure by Pacific staghorn sculpins to the extent that the chum salmon quickly outgrew their vulnerability to their predators, as recorded in other studies (Parker 1971; Patten 1977).

Healey (1982) reported a size-dependent mortality of juvenile chum salmon in the Nanaimo River estuary, BC. Parker (1971) found that predation of young chum by juvenile coho salmon was biased towards the smaller individuals, but because the daily rate of growth of these prey (1.4%) was higher than that of the predator (0.7%) the chum salmon outgrew this predator and became less vulnerable. However, Fresh and Schroder (1987) found that predation by rainbow trout on newly-emerged chum salmon was not selective for size nor related to their abundance, a result that most probably reflects upon the rudimentary escape behaviors of such young fish. The issue of size selectivity in the wild, when populations of prey species occur together, was investigated by Hargreaves and LeBrasseur (1985, 1986) in Masset Inlet, BC. They found that sub-adult coho salmon selectively preyed on pink salmon even when chum salmon were smaller and more abundant, thereby suggesting that the species of prey may be more important than prey size when predators are feeding among populations of mixed prey species.

### Prey escape speed

The maximum swim speed  $(V_{max})$  of the chum salmon used in the deep-water predator challenge tests was determined to increase during the early part of the experimental period in relation to their increasing size. These determinations were made on randomly selected fish and, therefore, the individuals may have been from either the treated or the

control group within the respective experimental test. There was no obvious difference in  $V_{max}$  among the treatments that inferred a reduced performance of one treatment group over another. Accordingly, it was impossible to discern from any of these observations whether or not any of the treatments affected the maximum swim speed of treated fish relative to that of the corresponding control groups.

Escape from predators has been examined by various researchers in relation to the size of fish and their burst speed, and it is considered by Taylor and McPhail (1985) that the "fast start" response that occurs within a fraction of a second may be closely associated with escape or capture. There is, though, a related importance of speed and maneuverability (Howland 1974). In a study by Taylor and McPhail (1985), the burst speed of juvenile coho salmon was determined to be greatest for the larger individuals and differences of up to 80% in swim performance were recorded within the first 0.03 s. This size-mediated difference in burst speed (approximately 19 body lengths  $\cdot s^{-1}$ ) was subsequently reflected in the predation of smaller rather than larger individuals of the test population, thus providing a causative factor for the differential mortality of juvenile salmon in the wild and the selection of smaller individuals. Bams (1967) also revealed a correlation between the swim performance of different sizes of sockeye salmon fry and the greater vulnerability to predation of the smaller fish which swam slower. Brett (1971b) determined how the maximum sustained swim speed is affected by the growth of juvenile salmon and that burst speed, which is virtually independent of temperature, is primarily an anaerobic activity that will incur an oxygen debt and lead to fatigue in the fish (Brett 1964, 1965, 1967). Repeated bursts of speed to escape predation will lead to fatigue, and it is possible that fish whose swim performance is compromised by stressors will more readily succumb to predation than those individuals not so affected.

In our experiments, we found a differential predation on the similar-sized treated chum salmon versus the control fish, for groups previously exposed to T+130 and/or T+120, but not for T+115. These findings suggest that among other effects, it is possible that the prior exposure to a combination of 20.7 °C seawater and DGS  $\geq$ 120% exerted an influence on swim performance (maximum burst speed) and thereby contributed to the selective capture of such treated individuals by the predators.

### **VULNERABILITY OF CHUM SALMON TO PREDATION**

The significantly increased vulnerability to predation of the groups of chum salmon exposed to T+120 in the deep-water challenge tests, as well as that for those groups exposed to T+120 or T+130 in the shallow-water tests, signifies a performance and/or sensory deficit of individuals within these (i.e., temperature, 20.7 °C, TGP,  $\geq$ 120%) treated groups of fish. The nature and cause of these deficits was not determined but may be inferred, in part, by reference to other studies and findings, and perhaps also from the results of the examination of the fish prior to the predator challenge experiments. In most studies that have investigated the susceptibility of fish to predation following exposure to stressful circumstances, the researchers have not separated performance deficits from sensory deficits (Schreck et al. 1997). We consider that the treated chum salmon that were most vulnerable to predation during our experiments most probably incurred a combination of both deficits: elevated temperature has the potential to reduce fish performance (e.g. Brett 1971b regarding swim performance) and dissolved gas supersaturation (DGS) the potential to affect both sensory function and performance (e.g. Weber and Schiewe 1976 regarding lateral line function; Schiewe 1974 regarding swim performance).

# **Predator efficiency**

An indication of the impaired performance of the treated chum salmon is inferred from an examination of the data relating to the efficiency with which rockfish captured their prey. The rockfish were less efficient at capturing the T+115 and control prey, than they were in capturing the T+120 or T+130 and control fish [ANCOVA revealed, however, a significant difference (p < 0.1) only for the capture success between the T+115 and T+120 groups]. Although the predators made more attacks on the T+115 group (including the control prey), their success at capturing these chum salmon was less than that with the groupings of T+120 and controls, or T+130 and controls.

The ratio of attacks to captures indicates the relative efficiency with which the rockfish captured the chum salmon, despite the growth of the prey over the experimental period. The predators were most efficient in their consumption of chum salmon exposed to T+120 followed by T+130 and T+115 in the respective predator challenge tests, and the corresponding capture:attack ratios of 1:3.1, 1:3.3, and 1:5.8 correlate with the overall consumption data which reveal that the greatest consumption of treated fish was in the T+120 group followed by the T+130 and T+115 groups. These supportive data infer that the sublethal exposure of warm seawater and DGS  $\geq$ 120% produced a vulnerability in the chum salmon to predation. There is no evidence that the T+115 treatment caused a greater susceptibility of these fish to predation relative to controls.

Predator attacks on their prey are not always successful. Vinyard (1982) describes how the predation of Sacramento perch (*Archoplites interruptus*) on evasive prey was successful 55% of the time, but required four times as much energy per attack as did the always successful attacks on non-evasive prey. Naive predators quickly learned to adapt to feeding on the non-evasive prey after 10 - 20 encounters. These results contrast with those of Healey and Reinhardt (1995), who studied the predation of naive and experienced juvenile coho and chinook salmon to predation by sub-adult rainbow trout. In their experiments, there was no relationship between the time or number of attacks to capture prey and vulnerability; only prey size was significantly correlated with vulnerability. In Healey and Reinhardts' experiments, the average capture:attack ratios ranged from 1:6 to 1:10 for "healthy" prey, revealing a lesser efficiency than that of rockfish feeding on a population of control and potentially stressed juvenile chum salmon in the WCS. Brown et al. (1985) examined the effects of pentachlorophenol on the guppy (Poecilia reticulata) and their predation by largemouth bass, and determined that the predators were most efficient at capturing those prev previously exposed to the greatest level of the stressor. Thus the bass obtained the greatest energetic benefit from the selection of these individuals. As in our experiments with chum salmon, they determined that the predators chased the lowest treatment group most often and the highest treatment group the least but for the greatest energetic reward. The highest treatment group of fish were the easiest to capture and correspondingly received the fewest attacks. The authors considered that the "latency" of the prey was related to their ease of capture, a situation similar to that reported by Kruzynski and Birtwell (1994) and Kruzynski et al. (1994) in which the behavior of juvenile chinook salmon was affected by sublethal exposure to an anti-sapstain chemical, and in turn this related to increased vulnerability to predation. In the experiments with chum salmon, there was no obvious latency of individuals within the test populations, and the most noticeable feature of the attack by the predators was that it occurred more frequently on solitary versus schooled prey rather than on visibly (to us) debilitated individuals.

## **Combined stressors**

The physiological effects of multiple stressors have been shown to be cumulative (Olla et al. 1995), and as a consequence these authors suggest that relative to the effects of a single stressor, exposure to multiple stressors would result in a longer period to recover anti-predator evasion behaviors. The two experimental data sets that were removed because of the likelihood possibility of the additional stress caused by disease affecting the results exemplify the concern over multiple stressors. In both instances, there was a difference in the consumption of treated vs control fish that was substantially different from the mean results (0.3% - 6.5%), obtained for the combined data set with the different predator groups, and 40% and 23% more treated than control fish were consumed after exposure to T+115. Schreck (1981) comments that stress reduces the performance capacity of the individual due to the costs of trying to maintain homeostasis and it is the manifestation of this reduction in performance that has often resulted in increased debilitation and vulnerability of individuals to predation. Mesa et al. (1994) considered that the causes of the vulnerability of individual fish to predation was related to a failure of the fish's mechanisms to detect the predator, lapses in decision making, poor fast-start performance, inability to school effectively, and increased consciousness. Collectively they represent, or are related to, performance and sensory deficits.

## Temperature, DGS, and effects on performance

The temperature of 20.7 °C to which the juvenile chum salmon were exposed in these studies would have been expected to increase the metabolic functions of the fish (Brett 1971b). Because this temperature exceeded the laboratory-derived avoidance threshold

for 50% of fish and was approaching levels that had proven to be acutely lethal with prolonged exposure (Brett 1952; Birtwell et al. 2001), the potential to stress the fish was present, and, by extension, the potential to impair performance.

An example of the induction of stress though acute exposure to elevation in temperature is given by Wedemeyer (1973). He determined that a rapid (3 min) but sublethal increase in temperature from 10 °C to 20 °C imposed a greater stress on juvenile steelhead trout than on coho salmon. Juvenile chum salmon in our experiments experienced a more gradual increase in temperature to the test level of 20.7 °C (i.e., a change of 9.2 °C over ~30 min) which might nonetheless have been stressful in itself. The same is true for the relative rapid (i.e., a change of 9.2 °C over ~45 min) decline in temperature which each of the groups of treated fish (but not the controls) experienced before the predator challenge tests. The results of Wedemeyer (1973), although not specifically related, reveal the potential of a rapid rise in temperate, in itself, to impose stress. Lesser changes in temperature have been found to impair the performance of juvenile salmon. For example, Brett (1967) determined that there was a 4% reduction in the swim performance of 15 °C - acclimated sockeye salmon at 20 °C, and that as temperatures approached lethal levels there was a rapid decline in swimming ability and a reduction in time to fatigue (Brett 1971b). Optimum temperature for the cruising speed of juvenile sockeye and coho salmon was determined to be 15 °C and 20 °C respectively (Brett et al. 1958), perhaps reflecting the different tolerances of these species to elevated temperature (Brett 1952).

Elevated levels of TGP have been demonstrated to impair the swim performance of juvenile chinook salmon. Schiewe (1974) determined that the performance of these fish was reduced by exposure to 106% - 120% TGP (the highest examined). The studies by Schiewe (1974) involved sustained swim performance, and as such may have greater relevance to the effects of longer exposures to elevated levels of TGP. Burst speed however, which appears to be a critical factor in predator avoidance (Taylor and McPhail 1985), was found to be independent of temperature (Brett 1971b). However, the ability of a fish to perform repeated swim challenges has been shown to decrease following exposure to sublethal stressors (Farrell et al. 1998; Jain et al. 1998) and intense activity can result in delayed mortality hours after this exercise (Wood et al. 1983). In the presence of predators, such performance deficits might, depending on their severity, enhance the susceptibility of individuals to predation.

The causal mechanisms of performance and/or sensory deficits evident for the groups of fish exposed to T+120 or T+130 were not readily apparent in our studies with chum salmon. It is possible that predator attacks on individuals exposed to T+120 or T+130 eventually reduced the performance of these groups of chum salmon, making them easier to capture. Exposure of naive prey to predators has been demonstrated to stress the prey (Järvi 1990) and render them more vulnerable to capture (Olla and Davis 1989), but in the

presence of the combined thermal and DGS (≥120%) stressors, the cumulative effects on naive fish may have further increased the susceptibility of the chum salmon to predation. Fidler (1998a, b) examined mechanisms and symptoms of the death of juvenile chinook salmon exposed to elevated TGP. His results show that under certain conditions of DGS. blood and tissue TGP levels rise in the venous system of the heart, and eventually bubbles grow there. While these bubbles are small they are dislodged and move to the gills and begin to block blood flow. The process continues with a progressive increase in the bubbles in the gills and the heart and eventually the blockage is so great that the fish dies (hemostasis). Quite obviously, before these events reach the lethal level, a significant impairment of blood flow to the tissues (and especially the gills) would be anticipated to have a debilitating effect on swim performance caused by an impairment of oxygen transfer and potential systemic hypoxia, respiratory acidosis, and necrosis from the build up of toxic wastes (Newcombe 1974). Stroud and Nebeker (1976) report that the movement of bubbles within the fish ("cascading bubble effect") can be lethal, and relate this movement to sudden activity and muscle contraction. The sudden requirement to move quickly (such as in escape from a predator) dislodged bubbles from their nucleation sites and produced a cascading effect of bubbles in circulation, resulting in death. It is possible therefore, that the requirement for rapid movement to escape from a predator in the challenge tests may have resulted in the release of bubbles in those fish that had been exposed to DGS. Furthermore, those fish that utilized anaerobic burst speed to escape predation would have incurred an oxygen debt and become fatigued (Brett 1971b). The recovery of these fish would be hindered if tissue hypoxia occurred due to reduced blood flow caused by gas bubbles in the circulatory system (Newcombe 1974; Farrell et al. 1998). The absence of gas emboli in the gills of the chum salmon surviving exposure to DGS  $\leq$ 130% indicates that these fish were not in as debilitated a state as were those chinook salmon that Mesa and Warren (1997) found to be most susceptible to predation. Based on Fiddler's (1998) most recent work, the fish that we exposed to 130% TGP in the present studies would have been expected to die in minutes or hours if they had not been returned to waters at 100% air saturation levels prior to the predation trials. We obtained differential predation of the treated fish relative to the controls and it is most likely that the juvenile chum salmon exposed to the T+120 or T+130 treatments were debilitated from the exposure to these levels of DGS due to microbubbles forming within their circulatory system and tissues. This presumed physiological effect, together with the combined effects of elevated temperature would have contributed to an impaired ability of these fish to escape predators.

## Gas Bubble Trauma and sensory deficits

The presence of external signs of gas bubble trauma (GBT) in a certain (low) percentage of the treated groups of chum salmon might have been symptomatic of internal GBT such that these fish were potentially disadvantaged through impairment of their blood circulation and oxygen transport. It is also possible that there was some impairment of the lateral line function as indicated by the low incidence of gas emboli

and hemorrhage that was present in some of the treated fish. The lateral line, together with vision, plays an important role in the schooling of fish (Dijkgraff 1962). It also serves to detect and locate moving animals at short range, functions in the detection of underwater objects (Weber and Schiewe 1976), and is important in the transmission of a fright response when sudden velocity changes occur (Partridge and Pitcher 1980). Its function is, therefore, intimately associated with the ability of fish to sense others and the ability of both individuals and schools to escape predation. This importance is exemplified by the studies on steelhead trout by Weber and Schiewe (1976). They found that bubbles occurred in the lateral line of these fish after a 2-h exposure to 130% TGP, and that the ability of fish to respond to stimuli correspondingly diminished or disappeared. Total occlusion of the lateral line by gas emboli resulted in no response of the fish to the stimulus. Thus the emboli mechanically blocked the function of the lateral line, but this sensory loss was reversible on return of the fish to waters at air-saturated (i.e., 100%) TGP levels. Based on the low incidence and severity of signs of GBT in the lateral line of chum salmon from all treatments in our experiments, we consider that any potential impairment of sensory function was probably small. Nonetheless, this potential impairment cannot be discounted as a possible contributing factor in the increased vulnerability to selective predation evident in our studies for those fish exposed to treatments of T+130 and/or T+120.

#### Prey vulnerability due to DGS

The severity and incidence of signs of GBT in our experiments contrast with those determined by Mesa and Warren (1997) in their experiments that assessed the vulnerability of chinook salmon to predation after exposure to DGS at levels up to and including 130% TGP. The approximately 37-min post-exposure period preceding the examination of fish for signs of GBT, in which the treated groups of chum salmon were returned to a temperature proximal to the acclimation temperature (100% TGP), may have been sufficient for some (but not statistically different) reduction in the external signs of GBT. The transient nature of external signs of GBT following the cessation of exposure to DGS conditions has been reported (e.g. Coutant and Genoway 1968; Weber and Schiewe 1976), with the potential for a corresponding increase in the recovery of predator evasion capabilities (e.g. as occurred for juvenile salmon within 1 h for recovery from such stresses as handling (Mesa 1994; Olla et al. 1995) and descaling (Gadomski et al. 1994). One may have expected that the signs of GBT in chum salmon would have been more severe because of the longer exposure period and the higher temperature to which they were exposed relative to those conditions in the studies by Mesa and Warren (1997) and Fidler et al. (1999). However, our experimental conditions were different, and the period in which we permitted the fish to acclimate to cooler waters before they were exposed to the predators potentially contributed to a reduction in any overt signs of GBT. Thus, the chum salmon exposed to the predators may have been suffering less from the symptoms of GBT than were those chinook salmon which Mesa and Warren (1997) used in their experiments. Despite the use of potentially less stressed fish in our experiments, we nevertheless obtained results that indicated that the groups of fish treated by exposure

to T+130 for 12 h, and/or to T+120 for 24 h, were more vulnerable than control fish to predation. Such was not the case for the groups of fish treated by exposure to T+115 for 48 h, in which instance their ability to escape predators did not differ significantly from that of the corresponding control groups.

Mesa and Warren (1997) consider that fish showing signs of GBT similar to those that they found in fish exposed to 130% TGP (for 3.5 h), regardless of their precise exposure history, may be more vulnerable to predation. It is apparent that fish in that treatment group, which had gas emboli in the gills, were in an advanced debilitated state and therefore most probably highly susceptible to predation. The treated chum salmon which were exposed to T+130 in our predator challenge experiments did not display the same severity of signs of GBT as reported by Mesa and Warren (1997) for this level of DGS but were, nevertheless, selectively captured. While we concur with Mesa and Warren (1997) over the expected enhanced vulnerability to predation of fish showing severe signs of GBT, the results of our experiments revealed that an enhanced and significant vulnerability to predation for groups of chum salmon exposed to T+130 and/or T+120 treatments occurred before such severe external signs of GBT were observed. Mesa and Warren (1997) consider that the reasons for the enhanced vulnerability to predation of chinook salmon exposed to 130% TGP was most likely due to these fish having a significantly greater proportion of their lateral lines and gills occluded with gas bubbles than fish exposed to other treatments. These authors suggest that this debilitation could result in sensory and performance deficits. It is also plausible that gas bubbles within the circulatory system of the fish played a significant part in the debilitation of the chinook salmon that were more susceptible to predation. Furthermore, the seemingly enhanced sensitivity of the predation challenges with chum salmon over that obtained by Mesa and Warren (1997) may also relate to their use of different exposure temperatures and to the different populations of chinook salmon prey among years of experimentation, which, albeit from the same source, may well have comprised individuals with different sensitivities to the test conditions and, therefore, increased the variance in their data.

We did not observe the same frequency or severity of signs of GBT in chum salmon exposed to either T+120 or T+130, after longer exposure periods in each treatment group than those used by Mesa and Warren (1997), but still determined differential predation for these two treatments over controls using one or both types of experimental apparatus employed here. Accordingly, we now speculate that the most likely cause for the increased vulnerability of the chum salmon observed in our studies was related most to physiological problems caused by the presence of bubbles within the vascular system of the fish (Fidler et al. 1999) affecting performance, combined with the additional stressful effects of elevated temperature.

#### Prey vulnerability due to temperature

Studies that have examined the effect of temperature on the potential vulnerability of fish to predation have tended to focus on the effects of heat-shock exposures (6 °C - 14.5 °C increase) in relation to their movement into or through thermal discharge plumes

(Sylvester 1972; Coutant 1973; Yocum and Edsall 1974), or cold shock (6 °C - 11 °C decrease; Coutant et al. 1974; Griffith 1978) as may occur through the sudden elimination of a thermal discharge in winter. In each case in which the fish were exposed to heat shock, they were subsequently more vulnerable to predation than control fish. Coutant (1973) determined that juvenile chinook salmon and rainbow trout were selectively preyed upon by sub-adult rainbow trout when the exposure times to elevated temperatures exceeded a minimum duration which was 10% (chinook) and 20% (rainbow trout) of that exposure duration that caused obvious loss of equilibrium of 50% of a test population at that temperature. While shorter exposures made shocked fish less vulnerable, longer ones increased vulnerability. Even the lowest temperature tested (26 °C) induced significantly different predation after exposures of >64 min but not at exposures of <32 min, and at the highest shock temperature of 30 °C exposures >1 min produced a statistically significant result. Coutant (1973) determined that selective predation began at exposures about 10% of the median time to death in the range of 26 °C to 30 °C, temperatures much higher than those used in the experiments with chum salmon, and ones which would be expected to be lethal to juvenile chum salmon in minutes with prolonged exposure (Brett 1952). It is interesting to note that the temperature-shocked fish used by Coutant (1973) showed some recovery from heat effects when returned to waters at the initial holding temperatures for 30 min to 60 min prior to predation. The holding period increased by three to four times the exposure time required to produce statistically significant differences in predation. Because we chose to minimize any thermal shock (and thus lessen the effects of the thermal exposure) to the chum salmon by allowing for a brief (approximately 37 min) acclimation period after exposure to particular treatment conditions and prior to any predation challenge, there was the potential, therefore, for the partial recovery from the effects of the thermal (and elevated DGS) exposure. In this regard, some of the fish exposed to a higher temperature thermal shock in Coutant's (1973) experiments displayed abnormal behavior during the exposure and were unable to swim upright; a feature that was lessened upon return to cooler waters of the predation test, but one that nevertheless resulted in abnormal behavior (fish with unnatural posture at the water surface, disorientation, erratic bursts of swimming activity). Coutant (1973) remarks that fish displaying such behavioral abnormalities were selectively preved upon by the predatory fish in the experiment, and that poor escape performance may have rendered these fish, and others, more susceptible to capture by predators.

Juvenile chum salmon exposed in the present studies to elevated temperature and DGS were not seen to display any of the behavioral abnormalities identified by Coutant (1973). The selective predation of the fish exposed to treatments of T+130 and/or T+120, relative to that for the corresponding control groups, implies that the inducement of sensory and/or performance deficit(s) in these treatments need not be revealed through gross and overt behavioral changes. The tendency for the rockfish to attack more solitary prey than those in the school may well relate to the recognition by the predators of more subtle behavioral modifications and related performance deficits in the treated fish (TGP,

 $\geq$ 120%) which rendered these fish easier to capture. To this extent, the initial predation on solitary fish was similar to that recorded by Coutant (1973).

# SIGNIFICANCE OF RESULTS TO THE JUVENILE CHUM SALMON UTILIZING PORT MOODY ARM

The foregoing comments and the results of these studies have indicated the potential for juvenile chum salmon that are exposed to elevated temperature and DGS to be more susceptible to predation. The question remains whether the results of these laboratory studies have relevance to the field situation and in particular to Port Moody Arm which receives the discharge of heated cooling water from the BGS. Without knowledge of the precise exposure history of individual fish to the waters of Port Moody Arm and a clear understanding of the seasonal water quality conditions therein, it is a question that can be answered only by inference based on our knowledge of the effects of these variables, the ecology, physiology and behavior of the chum salmon.

The presence of juvenile chum salmon in estuarine and near-shore coastal waters is somewhat dependent on the timing of their emergence and their growth rate. Juvenile chum salmon have been recorded as early as January in the estuary of the Fraser River (Birtwell. I. K., DFO, West Vancouver, BC, unpublished data), but the majority of chum salmon typically enter estuarine waters in March (Healey 1982; Macdonald and Chang 1993), and peak in April and May thereafter decreasing as they slowly (3 - 5 km·d<sup>-1</sup>) migrate to the Pacific Ocean (Simenstad and Salo 1987), however, some researchers have recorded their presence in Georgia Straight in the fall (refer to Healey 1982). Thus it may be expected that this salmonid species will utilize Port Moody Arm for a period of about 5 months and that within this period, individuals may be resident there for days to weeks (Levy et al. 1979).

The strong tendency of juvenile chum salmon to occupy the uppermost part of the water column has been documented in previous studies (e.g. Mason 1974; Birtwell and Harbo 1980; Birtwell and Kruzynski 1989; Birtwell et al. 1998), and this trait has resulted in the death of individuals in locations where toxic concentrations of pulp mill effluent have occurred in surface waters (McGreer and Vigers 1983; Birtwell and Kruzynski 1989). Although fish had the choice to move deeper in the water column to avoid the potentially lethal conditions of pulp mill effluent, they did not do so continuously and as a consequence some individuals died. During the preliminary experiments in the WCS the chum salmon occupied warmer surface waters with DGS in the presence of rockfish predators. In laboratory experiments carried out in 1997 it was determined that juvenile chum salmon would feed in waters in which the temperatures were potentially lethal with prolonged exposure (Birtwell et al. 2001; Korstrom et al. 1998). The results of field experiments (Birtwell et al. 1998) revealed that between 5% and 93% of the caged juvenile chum salmon occupied the surface waters (0 - 1m) of Port Moody Arm at various locations even when the temperature exceeded the laboratory-derived 50% avoidance of

20.2 °C (Birtwell et al. 2001). Thus, the potential exists for juvenile chum salmon to be exposed to the ambient, yet potentially stressful conditions which occur within the uppermost surface waters of Port Moody Arm because of their inherent trait to utilize these waters. Studies in 1997 (Birtwell et al. 1998), revealed that during the early summer (July), not only was temperature elevated in surface waters at study sites (maximum range 22.5 °C - 24 °C) but so too was TGP (maximum range 110% - 125%) at sites >70m from the BGS thermal discharge. Data presented in Table 14 reveal that temperatures in the surface (0.5 m) of Port Moody Arm were often less than the 50% temperature avoidance threshold and TGP values ranged from 103% - 114%.

Although the results of this study reveal the overall increased vulnerability to predation of chum salmon exposed to 20.7 °C seawater and DGS, depending upon exposure period, only for those fish exposed for 24 h to 120% TGP, or for 12 h to 130% TGP (shallow-water greenling and sculpin challenge test only) was there a significant increase in susceptibility to predation relative to control fish that were not subjected to the combined stressors. Clearly, the potential exists for increased predation of chum salmon in Port Moody Arm as a consequence of exposure to elevated temperatures and TGP in instances where these conditions are reached or exceeded (with respect to duration of exposure and level of stressors). Such predation in nature would most probably be from numerous species, such as coho salmon (e.g. Hargreaves and LeBrasseur 1985), and others, including birds and mammals.

Under the worst-case scenario relevant to Port Moody Arm, at temperatures of 22.5 °C to 24 °C, and TGP levels as high as 125% (Birtwell et al. 1998; one of 390 measurements) juvenile chum salmon would be expected to be vulnerable to predation after an exposure period of minutes to hours (<24). The elevated temperature and high (≥120%) TGP would be expected to exert a debilitating effect in less time than that which occurred after exposure to 20.7 °C and 125% TGP. It is not unreasonable to anticipate that, under such extreme (and presently atypical) circumstances for the surface waters of the Arm, predation of juvenile chum salmon exposed to such conditions for a matter of hours would be increased more than 10% to 20% over normal predation events which typically result in high mortality during their early sea life. It is also possible that the effect of longer exposures of juvenile chum salmon to elevated levels of temperature and to levels of TGP below those which we used in our experiments, but which typically occurred in the surface waters of Port Moody Arm in 1997 (Birtwell et al. 1998), would also lead to an increased susceptibility to predation. Such increased susceptibility would be a function of the duration of exposure and the levels of the combined stressors whose effects could well exceed those manifest in our short duration experiments. Bax (1983) reports a juvenile chum salmon mortality of 31% - 46% over a 40-d period, but his estimate of mortality may be higher than expected because dyed fish, which may have been more susceptible to predation (Baker and Modde 1977), were used in this study. In this context, exposure to the combined indiscriminate stressors of high temperatures and

TGP have the potential to affect all members of the chum salmon population, and the effects of exposure will be additive to the typical discriminate effects of natural selective predation (Brett 1958). It has been documented that predatory fish accumulate in areas such as estuaries at the time of the downstream migration of juvenile salmon and consume significant numbers of them (Larsson and Larsson 1975; Mace 1983; Hvidsten and Møkkelgjerd 1987). It is possible that such associations of predators and prey operate in Port Moody Arm, as elsewhere, and that the imposition of potentially stressful conditions only exacerbates this natural predatory situation.

The additional effects of reduced growth rate of chum salmon at elevated temperature and TGP (Greenbank et al. 1998, 2001), and hence a reduced swim speed relative to individuals of a greater size, and a consequential increase in susceptibility to predation, combined with potential sensory and performance deficits, could render chum salmon more vulnerable to predation than in the absence of such conditions. However, offsetting these potential negative effects is the potential for the chum salmon to move deeper in the water column and thereby gain the benefit of increased hydrostatic pressure and an associated effective reduction in TGP. This, however, may only be a transient phenomenon (Dawley et al. 1976b), which in addition to providing some relief from the exposure to elevated TGP also results in a movement away from preferred habitat. The consequences of this are difficult to determine, but the presence of predatory fish at depth would indicate that this habitat shift by juvenile salmon could be hazardous. Some relief for juvenile chum salmon from any adverse effects caused by potentially stressful levels of temperature and/or TGP in the surface waters of Port Moody Arm would be obtained due to supersaturated levels of dissolved oxygen therein, which are known to reduce the effects of N<sub>2</sub>-supersaturated waters (Fidler and Miller 1997). In addition, fish moving out of the high temperature/DGS waters and into waters at lower DGS levels (but not those which were hypoxic) would be expected to regain their predator avoidance capabilities (e.g. swim performance) within hours.

We speculate that, for the following reasons, the effects of the BGS thermal discharge will have but a small effect on the population of chum salmon using Port Moody Arm: the short duration of time within which the surface waters of Port Moody Arm are likely to be at potentially stressful levels for juvenile chum salmon (and therefore increase their susceptibility to predation); the naturally-declining numbers of this species due to migration to the Pacific Ocean in the early summer; and the potentially enhanced growth rate (and expected reduction in predation pressure) of the main part of the population due to the expected attraction to, and occupation of, the thermally-enriched and metabolically-optimal water temperatures in spring time. It will, however, have an effect of predisposing to predation, those few remaining individuals that choose to occupy surface waters during the early summer for protracted periods of time.

## SUMMARY AND CONCLUSIONS

Previous studies determined that juvenile chum salmon would continue to occupy the surface waters of Port Moody Arm in the early summer even when temperatures and dissolved gases were potentially stressful to them with prolonged exposure. Because an increase in temperature also coincided with increases in dissolved gases in surface waters of the Arm during the late spring and early summer, we considered that the well being of juvenile chum salmon may, in some instances, be compromised at such times. It is well known that the effects of stress can lead to a reduced performance in fish and that the effects of an imposition of multiple stressors is cumulative. Both elevated temperature and dissolved gas supersaturation have the capacity to debilitate fish and accordingly a combination of these variables could potentially exert an indiscriminate and cumulative effect on the chum salmon that use Port Moody Arm prior to their migration to the Pacific Ocean.

Fish live in a competitive environment in which their survival is related to the maintenance of health and performance. Predators tend to attack those prey that are in sub-standard condition or those that are conspicuous, hence the effect of aquatic variables that render fish more conspicuous (e.g. by a change in behavior), or debilitate them so that they become easier to catch, are an obvious cause for concern.

To address the potential of the seasonally-elevated temperatures and dissolved gases in the surface waters of Port Moody Arm to affect juvenile chum salmon, we employed test methodology that focused upon their survival when challenged by predators. No one test can measure the vitality of a fish better than the survival of an individual after a life time under natural conditions, but shorter-term whole organism tests that have a direct link to the survival of the individual, and that at the same time are ecologically meaningful, have value in environmental assessments. Accordingly, we chose a predator challenge test to assess the fitness of juvenile chum salmon after exposure to the combined stressors of elevated temperature and dissolved gas supersaturation. In these experiments, groups of equal numbers of control chum salmon exposed continuously to seawater at cool temperatures (12.8  $\pm$  1.0 °C) and 100% Total Gas Pressure (TGP) as well as those which had been exposed to 20.7 °C seawater and 115%, 120% or 130% Total Gas Pressure (TGP) for 48 h, 24 h, or 12 h respectively, were exposed to the risk of predation from locally occurring rockfish, greenling, and sculpin in apparatus at the West Vancouver Laboratory. Temperatures above this level, and to a maximum of about 24 °C, and TGP values to a maximum of about 125%, had been recorded in the surface waters of Port Moody Arm in July 1997 (Birtwell et al. 1998).

The results of the predator challenge tests revealed the increased vulnerability of juvenile chum salmon to the combined stressors of elevated temperature and TGP. Statistical analysis of the data revealed that the salmon were significantly more vulnerable

to predation after exposures of 24 h and 12 h to 120% and 130% TGP, respectively, in the warm seawater. The predators fed more efficiently on these fish than they did on those exposed to 115% TGP for 48 h, and signs of the effects of exposure to TGP (i.e. Gas Bubble Trauma) were most evident in the groups that were most heavily preyed upon. These results indicated that the performance of the juvenile salmon was compromised by the T+120 and T+130 treatments they received, thereby rendering them more susceptible to predation. We deduced that this performance deficit was possibly associated with the effects of gas bubbles on the sensory capabilities of the fish, but more so to the effects of temperature and TGP on swim performance and a reduced ability to recover from fatigue brought on by predator attacks and the need for repetitive escape responses.

It is possible to speculate on the significance of these findings to the populations of juvenile chum salmon that use Port Moody Arm. Because the concomitant effects of elevated temperature and TGP increased the vulnerability of chum salmon to predation, the potential exists for an increase in mortality of those populations that use Port Moody Arm, in those circumstances where the duration of exposure to the combination of temperature and DGS in the surface waters is sufficiently stressful and debilitating as was evident here for the T+120 and T+130 treatments. The mortality of juvenile salmonids is typically high during their early sea life (40% to 50%) due primarily, it is considered, to size-selective (discriminate) predation. Because elevated temperatures and TGP have the potential to affect all members of a population, these water quality variables may be viewed as indiscriminate stressors whose effects are additive to those of the natural predation events. Thus the potential is present in Port Moody Arm for juvenile chum salmon to suffer increased predation during the late spring and early summer when elevated temperatures and TGP occur in combination at levels which reach and or exceed those which were shown in the present studies (i.e., T+130 and or T+120) to cause such an effect or exposure for a greater duration to lower, yet still elevated levels of temperatures and/or TGP. The magnitude of any effect on the population of chum salmon at these times will be diminished because of the natural decrease in numbers of chum salmon that occur in near shore marine waters during this same period in time. However, for those individuals that remain there may be instances where levels of elevated temperature and/or DGS might prove problematic to their well being given a sufficient period of exposure. Intuitively one may expect that these fish would avoid potentially harmful conditions in their environment, but the choices which we deem to be appropriate are not always those which are made by the fish. There are numerous examples of fish occupying sub-optimal waters and suffering as a consequence. In this regard the chum salmon could move deeper in the water column to obtain the benefit of increased hydrostatic pressure and thereby reduce the effects of TGP (a movement to 1-m depth is equivalent to a reduction of 10% TGP), and a reduction in temperature. Such movements would take the fish from their preferred surface water habitat, and it is likely that any such movement would be of risk to their survival (predators tend to be in deeper water). The behavior to occupy the surface waters is probably adaptive and has contributed to the perpetuation of the species. Because of this, it is unlikely that

movement to depth would override this seemingly strong innate behavior for protracted periods of time.

Because Port Moody Arm tends to be eutrophic, dissolved oxygen levels are frequently above air-saturated levels during day time. Elevated levels of dissolved oxygen have been found to reduce the effects of nitrogen supersaturation on fish. However, at night the potential exists for hypoxic conditions to occur in eutrophic waters due to respiratory requirements of phytoplankton. If this were the case, the hypoxic waters themselves could impair performance of fish thus negating the benefit of increased oxygen levels during daytime.

An effect of increased and rapid growth of chum salmon upon entry to seawater would probably enhance their chances of survival, for as they grow they are able to swim faster and become progressively less available to some predators. Increased surface water temperatures in Port Moody Arm during the spring time could enhance the growth rate of chum salmon assuming adequate food supplies were available. Thus the continuous operation of the BGS could promote the growth of the chum salmon through the addition of heated cooling water which would raise the temperature in Port Moody Arm to that which is in the metabolically-optimal range for the salmon at an earlier date than that which would occur under natural circumstances (intermittent operation, however, would be potentially stressful to the fish due to rapidly fluctuating ambient conditions). The net result of the increased growth could be an increased chance of survival.

In conclusion, it was determined that the combined effect of elevated temperature and DGS at levels similar to, and exceeding, those which have been recorded on occasion (maxima for surface waters) in Port Moody Arm during early summer, has in such instances, and over  $\leq 24$  h, the potential to increase the susceptibility of juvenile salmon to predation. It is speculated that the effect of any increased vulnerability to predation of the population of chum salmon that use the Arm will be minimal because of the natural emigration of the majority of this species of salmon from near shore marine habitats to the Pacific Ocean, that has occurred by this time. Those fish that remain in the waters of Port Moody Arm would be expected to incur additional predation pressure commensurate with the duration of their exposure to, and the respective levels of, the combined stressors of elevated temperature and dissolved gas supersaturation.

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Table 1. Summary of water quality data and barometric pressure during the exposure of juvenile chum salmon to elevated temperature and Total Gas Pressure in 1998.

		TREAT	TREATMENT	
PARAMETER	CONTROL	T + 115	T + 120	T + 130
Z	187	74	72	46
Barometric Pressure (mm Hg)	$762.8 \pm 2.5$	$763.8 \pm 2.8*$	$762.5 \pm 2.0$	$761.8 \pm 2.3*$
∆P (mm Hg)	5 ± 7	117±9*	$157 \pm 6*$ <sup>a</sup>	228 ± 6* <sup>b c</sup>
Total Gas Pressure (%)	$100.6 \pm 0.9$	$115.3 \pm 1.1 *$	$120.5 \pm 0.8 *$ <sup>a</sup>	129.9±0.7* <sup>bc</sup>
Dissolved Nitrogen (% air saturation)	$101.5 \pm 1.2$	$117.6 \pm 1.6*$	$122.8 \pm 1.3 * a$	132.0±1.2* <sup>bc</sup>
Dissolved Oxygen (% air saturation)	97.5 ± 4.6	$106.8 \pm 4.0*$	111.7±3.5* <sup>a</sup>	122.0±3.7* <sup>bc</sup>
Dissolved oxygen (mg·L <sup>-1</sup> )	$9.0 \pm 0.5$	$8.2 \pm 0.3*$	8.5±0.3* <sup>a</sup>	9.3 ± 0.3 * <sup>b c</sup>
O <sub>2</sub> /N <sub>2</sub> ratio	$0.96 \pm 0.05$	$0.91 \pm 0.04*$	$0.91 \pm 0.04*$	$0.92 \pm 0.03*$
Temperature (°C)	$11.4 \pm 0.9$	$20.7 \pm 0.2*$	$20.7 \pm 0.04*$	$20.6 \pm 0.2*$
Salinity (‰)	$28.5 \pm 0.8$	$28.4 \pm 0.6$	$28.3 \pm 0.8$	$28.7 \pm 0.5^{\circ}$
Note: Values are reported as the mean ± SD of all readings	През			

Note: Values are reported as the mean  $\pm$  SU of all reacings

 $\star$  Indicates significant difference (p < 0.05) between control and treatment values

 $^{\rm a}$  Indicates significant difference (p < 0.05) between T + 115 and T + 120

 $^{\rm b}$  Indicates significant difference (p < 0.05) between T + 115 and T + 130

 $^{\rm c}$  Indicates significant difference (p < 0.05) between T + 120 and T + 130

salmon.						
	EYE	GILL	LATI	LATERAL LINE	CAUDAL FIN	L FIN
TREATMENT	EXOPTHALMIA	GAS EMBOLI	GAS EMBOLI	PETECHIAL HEMORRHAGE	SUBCUTANEOUS EMPHYSEMA	PETECHIAL HEMORRHAGE
CONTROL	%0	N/A	0.2%	4.2%	%0	1.3%
	0/930	0	1/455	19/455	0/465	6/465
T + 115	0.6%	%0	2.7%*	17.3%*	5.6%*	15.0%*
	2/320	0/55	4/150	26/150	9/160	24/160
T + 120	1.3%*	%0	6.7%*	26.7%*	4.0%*	10.7%*
	4/300	0/52	10/150	40/150	6/150	16/150
T + 130	1.3%*	%0	27.1%*	27.7%*	9.0%*	15.2%*
	4/310	0/83	42/155	51/184	14/155	28/184
$T + 130 DEAD^{1}$	%0	100%	44.3%*	N/A	65.6%*	N/A
	0/122	49/49	27/61	0	40/61	0
$T + 130 PRE^{2}$	%0	3.4%	75.9%*	48.30%*	10.3%*	20.7%*
	0/58	1/29	22/29	14/29	3/29	6/29
$T + 130 POST^3$	3.3%*	%0	63.3%*	33.30%*	10.0%*	46.7%*
	2/60	0/30	19/30	10/30	3/30	14/30
* indicates significan	* indicates significant differences ( $p < 0.05$ ) in the occurrence of GBT signs between control and treatment groups	e occurrence of GB	3T signs between c	ontrol and treatment groups		

<sup>1</sup> represents fish that died during the 12 h exposure period to 130% TGP

<sup>2</sup> represents fish sampled at the end of the exposure period prior to reacclimation to air-equilibrated conditions

<sup>3</sup> represents fish sampled at the end of the exposure period after reacclimation to air-equilibrated conditions

Table 2. The incidence and percentage of fish showing signs of Gas Bubble Trauma in control and treated groups of juvenile chum salmon

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Table 3. Severity and prevalence of signs of Gas Bubble Trauma in control and treated groups of juvenile chum salmon.

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	FISH		-	OCCLUSION		
TREATMENT	EXAMINED	0%0	1 - 5%	6 - 25%	26 - 50%	> 50%
		INCID	INCIDENCE OF GAS	AS EMBOLI	EMBOLI IN LATERAL LINE	C LINE
CONTROL	455	454		0	0	0
T + 115	150	146	4	0	0	0
T + 120	150	140	10	0	0	0
T + 130	155	113	30	6	m	0
T + 130 (DEAD) <sup>1</sup>	61	34	21	9	0	0
		INCII	DENCE OF E	INCIDENCE OF EMPHYSEMA IN CAUDAL FIN	N CAUDA	L FIN
CONTROL	465	465	0	0	0	0
T + 115	160	151	n	3	n	0
T + 120	150	144	4	2	0	0
T + 130	155	141	12		-	0
T + 130 (DEAD) <sup>1</sup>	61	21	19	18	ę	0
<sup>1</sup> represents fish that died during the 12 h exposure period	ied during the 12	h exposure p	beriod			

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	WEIG	HT (g)	LENGT	Ή (mm)
n	TREATED	CONTROL	TREATED	CONTROL
60	$2.12 \pm 0.18$	$2.10 \pm 0.19$	64 ± 2	64 ± 2
60	$2.13 \pm 0.16$	$2.07 \pm 0.20$	$63 \pm 2$	$63 \pm 2$
60	$2.14 \pm 0.17$	$2.05 \pm 0.21$	$64 \pm 1$	$63 \pm 2$
60	$2.25^{*} \pm 0.33$	$2.03 \pm 0.29$	$65 \pm 3$	$63 \pm 2$
60	$2.97 \pm 0.45$	$3.06 \pm 0.49$	$70 \pm 3$	$70 \pm 4$
60	$3.05 \pm 0.46$	$3.14 \pm 0.41$	$70 \pm 3$	$70 \pm 4$
60	$3.27 \pm 0.54$	$3.31 \pm 0.42$	72 ± 4	$73 \pm 3$
60	$3.29 \pm 0.37$	$3.28 \pm 0.40$	72 ± 3	$72 \pm 3$
60	$3.38 \pm 0.49$	$3.53 \pm 0.43$	$73 \pm 4$	74 ± 3
60	$3.48 \pm 0.53$	$3.48 \pm 0.50$	$74 \pm 3$	$73 \pm 4$
60	$4.75 \pm 0.55$	$4.72 \pm 0.46$	81 ± 3	81 ± 3
60	$4.80 \pm 0.54$	$4.83 \pm 0.54$	82 ± 3	83 ± 3
60	$4.99 \pm 0.70$	$5.17 \pm 0.66$	83 ± 3	84 ± 3
60	$5.13 \pm 0.55$	$5.22 \pm 0.60$	$85 \pm 3$	85 ± 3
60	$5.15 \pm 0.63$	$5.12 \pm 0.67$	84 ± 3	84 ± 3
60	$5.15 \pm 0.72$	$4.97 \pm 0.52$	84 ± 4	84 ± 3
60	$5.16 \pm 0.55$	$4.99 \pm 0.62$	84 ± 3	83 ± 3
40	$5.37 \pm 0.68$	$5.37 \pm 0.63$	85 ± 4	85 ± 3
60	$5.79 \pm 0.66$	$6.00 \pm 0.56$	88 ± 3	88 ± 3
60	$6.20 \pm 0.60$	$6.16 \pm 0.64$	$90 \pm 3$	89 ± 3
60	$6.32 \pm 0.78$	$6.28 \pm 0.62$	$90 \pm 3$	91 ± 3
60	$6.35 \pm 0.68$	$6.36 \pm 0.72$	$90 \pm 3$	91 ± 3
60	$6.45 \pm 0.71$	$6.66 \pm 0.60$	91 ± 3	$92 \pm 3$
50	$6.61 \pm 0.82$	$6.83 \pm 0.69$	91 ± 4	$92 \pm 3$
50	$6.66 \pm 0.57$	$6.83 \pm 0.80$	91 ± 3	92 ± 3
50	$7.05^{*} \pm 0.80$	$7.51 \pm 0.85$	93* ± 4	$95 \pm 3$
50	$7.00 \pm 0.77$	$7.06 \pm 0.82$	93 ± 3	$93 \pm 4$
60	$7.00 \pm 0.98$	$7.25 \pm 0.81$	92 ± 4	94 ± 3
60	$7.03 \pm 0.87$	$7.25 \pm 0.76$	93 ± 4	$94 \pm 3$

Table 4. The mean ( $\pm$  SD) length and weight of juvenile chum salmon subjected to predation by kelp greenling and Pacific staghorn sculpin in shallow water raceways.

\* indicates significant difference (p < 0.05) between control and treated fish

Table 4. Cont'd.

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	WEIG	HT (g)	LENGT	'H (mm)
n	TREATED	CONTROL	TREATED	CONTROL
60 60 30 60 30 30 30 30 30 30 30 30 30 30 30 30 30	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$ \begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$

Table 5. Results of predation, over 60 min, by kelp greenling and Pacific staghorn sculpin, on 20 control, and 20 treated juvenile chum salmon that were exposed for 48 h to 20.7 °C seawater and 115% Total Gas Pressure.

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					C	CONSUMPTION	NOL				
	# C	# CONSUMED	ED	OF T(	OF TOTAL RELEASED	ASED	OF TOTAI	OF TOTAL CONSUMED		STATISTICS	ICS
#	Т	С	DIF	TREATED	CONTROL	TOTAL	TREATED	CONTROL	df	$\chi^{2}$	PROB.
	6	10	-	45%	50%	47.5%	47.4%	52.6%		0.05	0.82
7	14	10	4	70%	50%	60.0%	58.3%	41.7%	<del>ب</del> ـــز	0:67	0.41
ς	16	11	5	80%	55%	67.5%	59.3%	40.7%		0.93	0.34
4	7	Ś	7	35%	25%	30.0%	58.3%	41.7%		0.33	0.56
Ś	5	~	Ϋ́	25%	40%	32.5%	38.5%	61.5%		0.69	0.41
9	14	13	Π	70%	65%	67.5%	51.9%	48.1%		0.04	0.85
7	6	8	<b>,</b>	45%	40%	42.5%	52.9%	47.1%		0.06	0.81
8	13	15	4	65%	75%	70.0%	46.4%	53.6%	<b>,</b>	0.14	0.71
6	10	11		50%	55%	52.5%	47.6%	52.4%	-	0.05	0.83
10	10	6	-	50%	45%	47.5%	52.6%	47.4%	-	0.05	0.82
11	11	11	0	55%	55%	55.0%	50.0%	50.0%		00.00	1.00
12	11	15	4	55%	75%	65.0%	42.3%	57.7%		0.62	0.43
13	12	8	4	60%	40%	50.0%	60.0%	40.0%	,	0.80	0.37
14	7	15	ŝ	35%	75%	55.0%	31.8%	68.2%	<del>,</del> ,	2.91	°.09*
15	10	8	7	50%	40%	45.0%	55.6%	44.4%	Π	0.22	0.64
POOLED	158	157	1	52.67%	52.33%	52.50%	50.16%	49.84%	1	0.00	0.96
DIFFERENCE	CE			0.	0.3%		-	0.3%			
HETEROGENEITY	ENEITY								14	7.55	0.91
ANALYSIS OF	OF DIF	DIFFERENCE SCORES	E SCOF	LES (t-test)					14		0.94
ANALYSIS OF	OF DIF	DIFFERENCE	E SCORES		(Mann-Whitney U test)	t)					0.73

T = treated; C = Control; DIF = Difference score between # of treated and control fish consumed; PROB = probability \* indicates significant difference (p < 0.10) from random (50:50 treated:control)

hum salmon that were exposed for 24 h to 20.7 °C seawater and 120% Total Gas Pressure.
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						CONSUMPTION	NOI				
	# C	# CONSUMED	ED	OF T(	OF TOTAL RELEASED	ASED	OF TOTAL	OF TOTAL CONSUMED		STATISTICS	ICS
#	T	С	DIF	TREATED	CONTROL	TOTAL	TREATED	CONTROL	df	$\chi^{2}$	PROB.
	10	7	<i>т</i>	50%	35%	42.5%	58.8%	41.2%		0.53	0.47
0	16	12	4	80%	60%	70.0%	57.1%	42.9%		0.57	0.45
ŝ	14	8	9	70%	40%	55.0%	63.6%	36.4%		1.64	0.20
4	8	12	4	40%	60%	50.0%	40.0%	60.0%		0.80	0.37
Ś	10	7	÷	50%	35%	42.5%	58.8%	41.2%		0.53	0.47
9	14	7	Г	70%	35%	52.5%	66.7%	33.3%		2.33	0.13
7	12	11		60%	55%	57.5%	52.2%	47.8%	,1	0.04	0.84
8	14	14	0	70%	70%	70.0%	50.0%	50.0%		00.00	1.00
6	10	6		50%	45%	47.5%	52.6%	47.4%		0.05	0.82
10	13	12	<b></b>	65%	%09	62.5%	52.0%	48.0%	<b>ب</b> ــــب	0.04	0.84
11	9	10	4	30%	50%	40.0%	37.5%	62.5%		1.00	0.32
12	10	9	4	50%	30%	40.0%	62.5%	37.5%	<del>,</del>	1.00	0.32
13	9	9	0	30%	30%	30.0%	50.0%	50.0%		0.00	1.00
14	13	10	ŝ	65%	50%	57.5%	56.5%	43.5%		0.39	0.53
15	12	7	S	60%	35%	47.5%	63.2%	36.8%		1.32	0.25
POOLED	168	138	30	56.00%	46.00%	51.00%	54.90%	45.10%		2.94	0.09*
DIFFERENCE	CE			10	10.0%		· · ·	9.8%			
HETEROGENEITY	ENEITY	ı							14	7.37	0.92
ANALYSIS OF DIFFERENCE SCORES	OF DIF	FERENC	<b>JE SCOH</b>	RES (t-test)					14		0.03**
ANALYSIS	OF DIF	OF DIFFERENCE	CE SCORES	RES (Mann-V	(Mann-Whitney U test)	t)					0.05**
T ++00+04		outrol. DIE	$\Pi = D: \mathcal{R}_{\text{constant}}$		hotmoon # of	tracted and	control fich of	or source hotseen # of twoodod and control fich community DD/D = wrohobility	4044	ability	

T = treated; C = Control; DIF = Difference score between # of treated and control fish consumed; PROB = probability \* indicates significant difference (p < 0.10) from random (50:50 treated:control)

\*\* indicates significant difference (p < 0.05) between the number of control and treated fish consumed

					C	CONSUMPTION	NOI				
	# C(	CONSUMED	ED	OF TC	OF TOTAL RELEASED	ASED	OF TOTAL	OF TOTAL CONSUMED		STATISTICS	TCS
#	[	U	DIF	TREATED	CONTROL	TOTAL	TREATED	CONTROL	df	$\chi^{2}$	PROB.
	11	9	5	55%	30%	42.5%	64.7%	35.3%	,l	1.47	0.23
7		9	2	55%	30%	42.5%	64.7%	35.3%	,(	1.47	0.23
m	9	9	0	30%	30%	30.0%	50.0%	50.0%	-	0.00	1.00
4	10	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	7	50%	40%	45.0%	55.6%	44.4%		0.22	0.64
5	10	9	4	50%	30%	40.0%	62.5%	37.5%		1.00	0.32
9	9	9	0	30%	30%	30.0%	50.0%	50.0%	,1	0.00	1.00
7	12	9	9	60%	30%	45.0%	66.7%	33.3%		2.00	0.16
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	10	6		50%	45%	47.5%	52.6%	47.4%		0.05	0.82
6	∞	7	1	40%	35%	37.5%	53.3%	46.7%		0.07	0.80
10	8	6	 I	40%	45%	42.5%	47.1%	52.9%		0.06	0.81
11	10	12	-2	50%	60%	55.0%	45.5%	54.5%	,	0.18	0.67
12	12	12	0	60%	60%	60.0%	50.0%	50.0%		00.00	1.00
13	13	11	7	65%	55%	60.0%	54.2%	45.8%	r1	0.17	0.68
14	12	10	7	60%	50%	55.0%	54.5%	45.5%		0.18	0.67
15	11	٢	4	55%	35%	45.0%	61.1%	38.9%	1	0.89	0.35
POOLED	150	121	29	50.00%	40.33%	45.17%	55.35%	44.65%	1	3.10	0.08*
DIFFERENCE	Ш			9.	9.7%			0.7%			
HETEROGENEITY	VEITY								14	4.71	0.99
ANALYSIS OF DIFFERENCE SCORES (t-test)	JF DIF	FERENC	CE SCOI	RES (t-test)					14		0.01**
ANALYSIS C	OF DIF	DIFFERENCE		RES (Mann-1	SCORES (Mann-Whitney U test)	st)					0.01**

Table 7 Results of predation over 60 min. by kelp greenling and Pacific staghorn sculpin, on 20 control, and 20 treated juvenile

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\*\* indicates significant difference (p < 0.05) between the number of control and treated fish consumed \* indicates significant difference (p < 0.10) from random (50:50 treated:control)

		ENTIR	ENTIRE TEST	TEST (FIRST'S MIN REMOVED)	AIN KEMUVED)
TREATMENT	_		# ATTACKS I	# ATTACKS BY ROCKFISH	
	Z	SOLITARY	SCHOOL	SOLITARY	SCHOOL
T + 115	5	45.6 ± 8.1	29.2 ± 12.2	24.2 ± 8.4	$21.6 \pm 13.0$
		61%	39%*	53%	47%
T + 120	9	$35.3 \pm 12.5$	$18.2 \pm 12.3$	$18.0 \pm 14.0$	$11.7 \pm 12.0$
		66%	34%*	61%	39%
T + 130	Ś	$36.2 \pm 14.0$	23.4 ± 6.9	$21.2 \pm 17.4$	16.6 ± 8.5
		61%	39%*	56%	44%
COMBINED	16	38.8 ± 12.0	23.3 ± 11.2	$20.9 \pm 13.0$	$16.3 \pm 11.3$
DATA		63%	37%*	56%	44%
* indicates significant differences ( $p < 0.05$ ) between % strikes by predators on schooling vs. solitary fish	ifferences (n	< 0.05) between % st	trikes by predators on	schooling vs. solitary f	ìsh

TISII SUILLAL D , N UII SUIUUIIUS 2 preuato. % SUTIKES UY nerweell indicates significant diriterences (p

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Table 9. Efficiency of rockfish when attacking populations comprised of control and treated juvenile chum salmon that were exposed to 20.7 °C seawater and elevated Total Gas Pressure.

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			PER	PER PREDATOR			EFFICIENCY
TREATMENT	TREATMENT # PREDATORS # ATTACKS	# ATTACKS	# CAPTURES	# MISSES	% ATTACKS	% MISSES	% ATTACKS % MISSES CAPTURES:ATTACKS
115%	$5.2 \pm 0.4$	14.6 ± 4.3	$2.5 \pm 0.7$	12.2 ± 4.3	17.0	83.0	1:5.8
120%	5.2 ± 0.4	$10.3 \pm 3.7$	3.3 ± 0.7	7.0 ± 3.6	31.8*	68.2	1:3.1
130%	5.5 ± 0.5	$10.7 \pm 4.0$	$3.2 \pm 1.0$	7.5 ± 3.7	30.0	70.0	1:3.3
NOTE: values are	NOTE: values are represented as mean $\pm$ SD (n = 6)	± SD (n = 6)					

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\* indicates a significant (p < 0.10) difference in % capture success relative to the 115% TGP treatment group

WEIG	HT (g)	LENGT	H (mm)
TREATED	CONTROL	TREATED	CONTROL
$2.64 \pm 0.37$ $2.78 \pm 0.41$ $3.81 \pm 0.47$ $4.48 \pm 0.43$ $4.72 \pm 0.50$ $5.36 \pm 0.61$ $5.43 \pm 0.60$ $5.63 \pm 0.62$ $6.17 \pm 0.67$ $6.95^* \pm 0.70$ $6.86 \pm 0.81$ $11.85 \pm 1.39$ $12.59 \pm 1.65$ $12.76 \pm 1.67$ $13.51 \pm 1.36$	$2.64 \pm 0.36  2.77 \pm 0.49  3.83 \pm 0.42  4.42 \pm 0.49  5.30 \pm 0.66  5.65 \pm 0.54  5.67 \pm 0.67  6.28 \pm 0.73  6.55 \pm 0.85  7.17 \pm 0.75  12.09 \pm 1.66  12.42 \pm 1.97  12.25 \pm 2.07  13.47 \pm 1.83$	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$68 \pm 3$ $68 \pm 4$ $75 \pm 3$ $79 \pm 3$ $79 \pm 3$ $85 \pm 3$ $87 \pm 3$ $87 \pm 3$ $90 \pm 3$ $91 \pm 3$ $94 \pm 3$ $109 \pm 4$ $110 \pm 5$ $111 \pm 6$ $116 \pm 5$
$13.10 \pm 1.67 \\ 13.37 \pm 1.59 \\ 14.26^{\#} \pm 1.54$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$110 \pm 4$ $115 \pm 5$ $117 \pm 4$ $119 \pm 4$	$115 \pm 5$ $115 \pm 5$ $115 \pm 5$ $119 \pm 5$

Table 10. The mean ( $\pm$  SD) length and weight of 40 control and 40 treated juvenile chum salmon subjected to predation by rockfish in the deep water aquarium.

\* indicates significant difference (p < 0.05) between control and treated fish

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					Ŭ	CONSUMPTION	N				
	# C(	# CONSUMED	ED	OF T	OF TOTAL RELEASED	VSED	OF TOTAL CONSUMED	CONSUMED	S	STATISTICS	ICS
#	Е	C	DIF	TREATED	CONTROL	TOTAL	TREATED	CONTROL	df	. χ <sup>2</sup>	PROB.
	10	∞	7	50%	40%	45.0%	55.6%	44.4%	-	0.22	0.64
7	5	4		25%	20%	22.5%	55.6%	44.4%	<b>,</b> i	0.11	0.74
ŝ	6	9	ŝ	45%	30%	37.5%	60.0%	40.0%	,(	0.60	0.44
4	9	6	<u>.</u>	30%	45%	37.5%	40.0%	60.0%		0.60	0.44
5	2	7	Ś	35%	10%	22.5%	77.8%	22.2%		2.78	0.10*
9	4	7	<del>د</del> .	20%	35%	27.5%	36.4%	63.6%	<del>,</del> 1	0.82	0.37
POOLED	41	36	5	34.17%	30.00%	32.08%	53.25%	46.75%		0.32	0.57
DIFFERENCE	Ц			4.	4.2%			6.5%			
HETEROGENEITY ANALYSIS OF DIFFERENCE SCORES ANALYSIS OF DIFFERENCE SCORES	NEIT' OF DII OF DII	Y FFEREN FFEREN	ICE SCC ICE SCO		(t-test) (Mann-Whitney U test)				ŚŚ	4.83	0.44 0.56 0.60

\* indicates significant difference (p < 0.10) from random (50:50 treated:control)

Table 11. Results of predation by rockfish on 20 control, and 20 treated juvenile chum salmon that were exposed for 48 h to 20.7 °C

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Table 12. Results of predation by rockfish on 20 control, and 20 treated juvenile chum salmon that were exposed for 24 h to 20.7 °C seawater and 120% Total Gas Pressure.

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					Õ	CONSUMPTION	Z				
	# C	# CONSUMED	ΕD	OF T	OF TOTAL RELEASED	ASED	OF TOTAL CONSUMED	CONSUMED	Š	STATISTICS	ICS
#	Т	C	DIF	TREATED	CONTROL	TOTAL	TREATED	CONTROL	df	, χ <sup>2</sup>	PROB.
	11	7	4	55%	35%	45.0%	61.1%	38.9%		0.89	0.35
7	13	6	4	65%	45%	55.0%	59.1%	40.9%		0.73	0.39
ŝ	11	9	5	55%	30%	42.5%	64.7%	35.3%	<del>,</del>	1.47	0.22
4	12	9	9	60%	30%	45.0%	66.7%	33.3%	<del>,</del>	2.00	0.15
5	8	5	3	40%	25%	32.5%	61.5%	38.5%		0.69	0.40
9	٢	9		35%	30%	32.5%	53.8%	46.2%	,i	0.08	0.78
POOLED	62	39	23	51.67%	32.50%	42.08%	61.39%	38.61%		5.24	0.02*
DIFFERENCE	NCE			19.	19.2%		22.	22.8%			
HETEROGENEITY ANALYSIS OF DIF ANALYSIS OF DIF	GENEIT' S OF DI S OF DI	Y FFEREN FFEREN	ICE SCO	HETEROGENEITY ANALYSIS OF DIFFERENCE SCORES (t-test) ANALYSIS OF DIFFERENCE SCORES (Mann-Whitney U test)	'hitney U test)				s s	0.65	0.99 0.00** 0.03**
T = treated;	11	ontrol; E	C = Control; DIF = Difference	II	etween # of tre	sated and contr	score between # of treated and control fish consumed; PROB = probability	ed; PROB = pr	obabili	ty	

\*\* indicates significant difference (p < 0.05) between the number of control and treated fish consumed \* indicates significant difference (p < 0.10) from random (50:50 treated:control)

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CONTRACTOR NAME

					Č	CONSUMPTION	Z				
, <u> </u>	# C	# CONSUMED	ED	OF T	OF TOTAL RELEASED	VSED	OF TOTAL CONSUMED	CONSUMED	Ň	STATISTICS	ICS
#	H	U	DIF	TREATED	CONTROL	TOTAL	TREATED	CONTROL	df	, X <sup>2</sup>	PROB.
	12	L	5	60%	35%	47.5%	63.2%	36.8%	Ţ	1.32	0.25
2	15	7	~	75%	35%	55.0%	68.2%	31.8%	,'	2.91	0.09*
m	5	4	1	25%	20%	22.5%	55.6%	44.4%	<del>،</del>	0.11	0.74
4	10	10	0	50%	50%	50.0%	50.0%	50.0%	<b>,</b> `	0.00	1.00
5	10	12	-7	50%	60%	55.0%	45.5%	54.5%		0.18	0.67
9	٢	2	0	35%	35%	35.0%	50.0%	50.0%	<b>,</b> (	0.00	1.00
POOLED	59	47	12	49.17%	39.17%	44.17%	55.66%	44.34%		1.36	0.24
DIFFERENCE	NCE			10.	10.0%		11.	11.3%			
HETEROGENEITY ANALYSIS OF DIFFERENCE SCORES ANALYSIS OF DIFFERENCE SCORES	GENEIT S OF DI S OF DI	Y FFEREN FFEREN	VCE SCC		t-test) Mann-Whitney U test)				s s	3.2	0.67 0.25 0.27

T = treated; C = Control; DIF = Difference score between # of treated and control fish consumed; PROB = probability \* indicates significant difference (p < 0.10) from random (50:50 treated:control)

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DATE	SITE	DEPTH (m)	TEMP (°C)	TEMP (°C) SALINITY (‰)	TGP (%)	DO <sub>2</sub> (% sat)	N <sub>2</sub> & Ar (% sat)	$O_2:N_2$
Jun-25		0.5	15.27	16.2	107.5	110	109.53	1.01
Jun-25		1.5	15.26	17.0	107.7	101	111.44	0.90
Jun-25	1	2.5	14.66	19.6	103.8	78	111.99	0.70
Jun-25	1	3.5	14.01	20.0	103.5	77	112.16	0.69
Jun-25	1	4.5	12.28	22.3	102.0	74	111.46	0.66
Jun-25	*****	5.5	12.48	22.7	101.6	69	112.01	0.62
Jun-26		0.5	15.82	16.3	106.5	109	108.39	1.01
Jun-26	1	1.5	16.43	18.4	107.3	101	111.10	0.91
Jun-26	*4	2.5	16.07	19.5	104.3	94	109.07	0.86
Jun-26	1	3.5	13.76	22.2	104.2	81	112.35	0.72
Jun-26	1	4.5	12.93	22.6	101.4	84	108.01	0.77
Jun-26	-	5.5	12.27	23.0	101.6	80	108.98	0.73
Jul-08	1	0.5	16.11	20.3	105.9	113	103.55	1.09
Jul-08	1	1.5	15.25	21.4	107.8	105	108.31	0.97
Jul-08	1	2.5	14.31	22.0	105.1	94	108.12	0.87
Jul-08		3.5	14.29	22.2	105.1	89	109.29	0.81
Jul-08	*****	4.5	13.10	22.6	102.4	84	106.39	0.79
Jul-08	<b></b> 1	5.5	12.29	22.9	101.2	82	105.65	0.78

Table 14. Cont'd.	Cont'd.							
DATE	SITE	DEPTH (m)	TEMP (°C)	SALINITY (‰)	TGP (%)	DO <sub>2</sub> (% sat)	N <sub>2</sub> & Ar (% sat)	O <sub>2</sub> :N <sub>2</sub>
Jul-09	-	0.5	15.65	17.5	107.2	116	103.75	1.12
Jul-09	-	1.5	14.03	20.2	106.2	104	106.29	0.98
Jul-09	1	2.5	13.71	20.8	105.9	96	107.99	0.89
Jul-09	1	3.5	14.08	21.4	105.1	92	108.42	0.85
Jul-09		4.5	14.23	21.7	104.3	91	107.70	0.84
Jul-09	1	5.5	13.62	21.8	104.1	89	107.66	0.83
Jul-10	-	0.5	15.59	16.7	108.6	119	105.76	1.13
Jul-10	4	1.5	15.11	18.1	108.4	113	106.85	1.06
Jul-10	1	2.5	15.76	20.7	107.1	66	108.48	0.91
Jul-10	*4	3.5	15.16	21.2	106.4	98	108.76	0.90
Jul-10	fd	4.5	15.07	21.5	105.2	98	107.25	0.91
Jul-10	1	5.5	14.43	21.6	105.1	95	107.90	0.88
Jul-11	1	0.5	15.18	15.0	109.7	131	104.35	1.26
Jul-11	1	1.5	16.93	17.5	111.7	135	104.82	1.29
Jul-11	+4	2.5	16.03	19.1	108.1	113	106.85	1.06
Jul-11	<b>F</b>	3.5	16.01	20.0	107.8	104	108.63	0.96
Jul-11	1	4.5	15.18	21.1	106.0	94	108.01	0.87
Jul-11	1	5.5	15.07	21.6	103.9	89	107.39	0.83

Note: Site 1 was the reference site and was located 250 m west of the Burrard Generating Station (BGS) thermal discharge point.

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DATE	SITE		TEMP (°C)	DEPTH (m) TEMP (°C) SALINITY (‰)	TGP (%)	DO <sub>2</sub> (% sat)	N <sub>2</sub> & Ar (% sat)	O <sub>2</sub> :N <sub>2</sub>
Jul-15		1.5	20.33	14.5	114.1	135	109.69	1.23
Jul-15		2.5	17.00	19.3	111.0	100	114.28	0.88
Jul-15	( <del>,</del>	3.5	16.52	19.8	108.1	66	109.97	06.0
Jul-15		4.5	15.58	20.0	104.7	98	106.14	0.92
Jul-15	( <del>,</del> 1	5.5	15.40	21.0	103.8	91	107.54	0.85
Jul-16		0.5	19.48	15.2	112.6	143	105.32	1.36
Jul-16	4	1.5	19.19	15.7	113.7	136	107.62	1.26
Jul-16	1	2.5	17.10	16.9	112.1	131	108.18	1.21
Jul-16	<b>[</b>	3.5	17.96	17.9	113.0	118	111.41	1.06
Jul-16	Ţ	4.5	17.64	18.5	109.5	113	108.11	1.05
Jul-16		5.5	17.09	19.1	107.8	104	108.66	0.96
Jul-17		0.5	19.31	15.6	112.2	133	107.08	1.24
Jul-17		1.5	19.21	15.8	113.0	133	108.41	1.23
Jul-17	•	2.5	18.43	17.0	113.4	124	111.41	1.11
Jul-17		3.5	16.89	17.5	112.6	112	112.84	0.99
Jul-17		4.5	17.16	18.2	108.9	108	109.02	0.99
Jul-17	<b>,</b>	5.5	16.75	18.5	107.9	107	107.99	0.99

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DATE	SITE	DEPTH (m)	TEMP (°C)	SITE DEPTH (m) TEMP (°C) SALINITY (‰)	TGP (%)	DO <sub>2</sub> (% sat)	$N_2$ & Ar (% sat)	O <sub>2</sub> :N <sub>2</sub>
Jul-18	1	0.5	19.70	16.3	111.4	133	106.07	1.25
Jul-18	+4	1.5	19.45	16.5	112.5	118	111.15	1.06
Jul-18		2.5	18.68	17.7	111.4	114	110.14	1.04
Jul-18	1	3.5	18.11	18.5	107.8	100	109.45	0.91
Jul-18		4.5	15.20	19.0	104.6	95	107.12	0.89
Jul-18	1	5.5	15.00	19.7	103.3	96	104.87	0.92
Aug-19		0.5	19.55	23.0	109.8	125	105.58	1.18
Aug-19		1.5	19.32	23.1	111.0	132	105.72	1.25
Aug-19	<b></b>	2.5	16.34	23.6	104.5	102	105.47	0.97
Aug-19	Ц	3.5	15.81	23.7	103.7	93	106.34	0.87
Aug-19	+4	4.5	15.63	23.8	101.4	89	104.10	0.85
Aug-19		5.5	15.55	23.8	101.4	86	105.04	0.82
Aug-20	<del>,</del>	0.5	18.67	23.3	112.0	130	106.81	1.22
Aug-20	<del>,</del>	1.5	18.52	23.3	111.9	129	106.66	1.21
Aug-20	1	2.5	17.76	23.5	110.4	117	108.00	1.08
Aug-20	1	3.5	16.46	23.8	106.9	102	108.09	0.94
Aug-20	1	4.5	16.26	23.8	104.9	98	106.48	0.92
Aug-20		5.5	15.65	23.9	104.0	96	105.62	0.91

Note: Site 1 was the reference site and was located 250 m west of the Burrard Generating Station (BGS) thermal discharge point.

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Table	

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DATE	SITE	DEPTH (m)	TEMP (°C)	TEMP (°C) SALINITY (‰)	TGP (%)	DO <sub>2</sub> (% sat)	N <sub>2</sub> & Ar (% sat)	O <sub>2</sub> :N <sub>2</sub>
Aug-21		0.5	18.47	23.2	107.2	114	105.80	1.08
Aug-21	+4	1.5	17.77	23.5	107.8	115	106.01	1.08
Aug-21		2.5	17.06	23.7	105.1	102	105.91	0.96
Aug-21	7	3.5	15.92	23.9	103.8	96	105.70	0.91
Aug-21	Π	4.5	15.58	23.9	102.1	97	103.22	0.94
Aug-21	<b>,</b>	5.5	15.53	23.9	102.2	66	102.46	0.97
Aug-22		0.5	17.97	23.5	108.8	119	105.49	1.13
Aug-22	1	1.5	17.69	23.6	109.9	115	107.99	1.06
Aug-22	1	2.5	17.39	23.7	106.5	108	106.09	1.02
Aug-22	<b>****</b>	3.5	17.06	23.7	104.2	104	103.69	1.00
Aug-22	Ţ	4.5	16.37	23.8	102.9	95	104.42	0.91
Aug-22	<b>—</b>	5.5	15.62	24.0	102.1	93	103.74	0.90
8-Jul	2	0.5	17.49	21.8	108.8	98	111.60	0.88
8-Jul	2	1.5	17.22	22.5	110.5	97	113.63	0.85
8-Jul	7	2.5	18.06	22.7	110.0	94	113.75	0.83
8-Jul	2	3.5	16.91	23.2	109.9	94	114.07	0.82
8-Jul	7	4.5	15.25	23.0	108.4	88	114.09	0.77
8-Jul	7	5.5	13.09	23.3	105.7	82	111.77	0.73
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Note: Site 1 was the reference site and was located 250 m west of the Burrard Generating Station (BGS) thermal discharge point. Site 2 was located 70 m east of the BGS thermal discharge point.

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Table

DATE	SITE	DEPTH (m)	TEMP (°C)	SITE DEPTH (m) TEMP (°C) SALINITY (‰)	TGP (%)	DO <sub>2</sub> (% sat)	N <sub>2</sub> & Ar (% sat)	O <sub>2</sub> :N <sub>2</sub>
Jul-09	5	0.5	17.97	20.3	108.8	98	111.08	0.88
Jul-09	7	1.5	18.36	20.7	110.9	94	115.37	0.81
Jul-09	7	2.5	18.19	21.9	110.8	89	115.83	0.77
Jul-09	7	3.5	16.56	22.1	109.9	88	115.43	0.76
Jul-09	7	4.5	14.96	21.7	107.0	87	112.92	0.77
Jul-09	7	5.5	14.41	22.3	106.4	83	112.99	0.73
1,11_10	C	5 0	1761	19.2	110.9	106	111.67	0.95
Jul-10	10	1.5	18.95	19.8	112.2	100	115.04	0.87
Jul-10	7	2.5	18.84	20.2	112.6	98	116.51	0.84
Jul-10	7	3.5	18.72	21.6	112.8	94	117.49	0.80
Jul-10	2	4.5	16.27	21.8	109.3	89	115.27	0.77
Jul-10	5	5.5	14.89	22.0	107.7	84	113.71	0.74
Jul-11	7	0.5	16.14	14.1	109.4	129	104.15	1.24
Jul-11	2	1.5	17.32	17.6	110.8	117	108.84	1.08
Jul-11	0	2.5	18.29	19.8	110.8	105	111.04	0.95
Jul-11	7	3.5	18.87	20.8	110.7	67	113.83	0.85
Jul-11	7	4.5	17.03	21.7	108.1	92	111.77	0.82
Jul-11	7	5.5	16.88	22.2	107.0	82	112.57	0.73
Note: Site 2	2 was loce	Note: Site 2 was located 70 m east of the B	he BGS thermal c	GS thermal discharge point.				

DATE	SITE	SITE DEPTH (m)		TEMP (°C) SALINITY (‰)	TGP (%)	$DO_2$ (% sat)	$N_2$ & Ar (% sat)	O <sub>2</sub> :N <sub>2</sub>
Jul-15	2	0.5	21.09	13.9	110.0	121	108.32	1.12
Jul-15	2	1.5	21.14	16.3	111.5	112	112.11	1.00
Jul-15	2	2.5	20.15	20.4	110.7	94	115.97	0.81
Jul-15	2	3.5	18.05	21.1	110.2	06	116.83	0.77
Jul-15	7	4.5	17.45	21.3	106.8	. 68	111.96	0.79
Jul-15	7	5.5	16.83	21.5	105.6	89	110.46	0.81
T <sub>11</sub> ]_16	C	50	20.91	17.0	114 3	127	111 60	1 14
Jul-16	1 (1	1.5	20.75	17.8	115.5	121	113.80	1.06
Jul-16	1 (1	2.5	20.94	18.5	115.2	119	114.45	1.04
Jul-16	7	3.5	21.28	18.6	114.8	111	115.27	0.96
Jul-16	7	4.5	19.29	19.4	112.5	105	115.55	0.91
Jul-16	7	5.5	17.77	20.1	110.3	95	113.52	0.84
Jul-17	7	0.5	21.50	17.2	115.1	120	114.41	1.05
Jul-17	7	1.5	21.77	17.4	115.8	117	115.92	1.01
Jul-17	0	2.5	21.48	17.8	115.4	113	115.53	0.98
Jul-17	0	3.5	20.46	18.1	114.2	111	116.04	0.96
Jul-17	2	4.5	19.99	18.3	112.4	116	111.70	1.04
Jul-17	0	5.5	18.88	19.4	110.5	96	114.07	0.84

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DATE	SITE	DEPTH (m)	TEMP (°C)	SALINITY (‰)	TGP (%)	$DO_2$ (% sat)	N <sub>2</sub> & Ar (% sat)	O <sub>2</sub> :N <sub>2</sub>
Jul-18	0	0.5	20.34	17.7	112.5	114	112.83	1.01
Jul-18	7	1.5	21.00	18.2	113.5	110	114.64	0.96
Jul-18	2	2.5	21.15	18.1	113.0	107	114.62	0.93
Jul-18	2	3.5	20.64	19.2	110.8	107	112.10	0.95
Jul-18	2	4.5	19.63	19.8	109.2	98	110.91	0.88
Jul-18	7	5.5	17.78	19.8	106.6	96	109.51	0.88
Aue-19	7	0.5	19.01	23.7	106.9	86	112.05	0.77
Aug-19	7	1.5	18.08	23.4	106.4	86	111.81	0.77
Aue-19	7	2.5	17.43	23.8	104.8	86	110.21	0.78
Aug-19	7	3.5	17.20	23.9	103.5	87	107.89	0.81
Aug-19	7	4.5	16.31	24.1	101.7	80	106.60	0.75
Aug-19	7	5.5	15.97	24.3	100.4	78	105.55	0.74
Aug-20	2	0.5	19.56	23.9	109.1	98	111.54	0.88
Aug-20	7	1.5	18.56	23.8	109.1	94	112.36	0.84
Aug-20	0	2.5	17.46	24.5	107.0	95	109.94	0.86
Aug-20	2	3.5	18.35	24.0	106.9	95	109.27	0.87
Aug-20	0	4.5	17.61	23.8	104.5	06	106.22	0.85
Aug-20	7	5.5	15.75	24.1	103.4	89	105.87	0.84

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	1.5 2.5 3.5 5.5 5.5	18.45 17.81 17.61 15.67		10/.0	91	110.98	0.82
	2.5 3.5 5.5 5.5	17.81 17.61 15.67	24.0	106.3	91	110.09	0.83
	3.5 5.5 5.5	17.61 15.67	24.0	104.8	91	107.92	0.84
	4.5 5.5	15.67	23.8	103.9	94	106.11	0.89
0 0 0	5.5		23.9	103.1	93	105.77	0.88
7 7		15.58	24.0	101.3	89	104.04	0.86
5	0.5	19.48	24.0	107.7	94	111.12	0.85
,	1.5	18.62	23.9	106.9	97	109.14	0.89
	2.5	17.48	23.8	105.9	93	108.75	0.86
2	3.5	16.42	24.3	104.6	94	107.26	0.88
2	4.5	16.03	24.1	103.4	93	105.75	0.88
2	5.5	15.10	24.2	102.1	06	105.18	0.86
Jul-08 3 0	0.5	16.06	17.2	104.7	112	103.54	1.08
Jul-08 3 1	1.5	16.82	19.9	106.7	102	107.67	0.95
Jul-08 3 2	2.5	16.78	20.9	107.4	93	110.68	0.84
Jul-08 3 3	3.5	14.63	22.6	104.2	81	109.32	0.74
Jul-08 3 4	4.5	13.67	22.9	101.7	75	107.76	0.70
Jul-08 3 5	5.5	12.68	23.1	100.0	73	106.30	0.69

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Site 3 was located 250 m east of the BGS thermal discharge point.

DATE	SITE	DEPTH (m)	TEMP (°C)	TEMP (°C) SALINITY (‰)	TGP (%)	DO <sub>2</sub> (% sat)	N <sub>2</sub> & Ar (% sat)	O <sub>2</sub> :N <sub>2</sub>
Jul-09	3	0.5	18.36	16.4	110.4	106	112.07	0.95
Jul-09	ε	1.5	16.35	20.4	112.0	104	114.35	0.91
Jul-09	С	2.5	16.62	21.0	108.5	96	111.27	0.86
Jul-09	С	3.5	16.26	21.3	108.3	92	112.64	0.82
Jul-09	С	4.5	15.59	21.4	106.2	91	109.94	0.83
Jul-09	÷	5.5	14.66	21.7	105.3	91	108.95	0.84
.Jul-10	(1)	0.5	16.98	17.3	114.4	139	108.04	1.29
Jul-10	ŝ	1.5	17.74	18.3	114.5	132	108.54	1.22
Jul-10	т	2.5	18.42	19.7	113.2	112	114.06	0.98
Jul-10	Ś	3.5	17.88	21.2	111.0	66	114.03	0.87
Jul-10	Ś	4.5	16.57	21.5	107.7	97	110.32	0.88
Jul-10	Ś	5.5	15.51	21.7	106.5	95	109.62	0.87
Jul-11	ŝ	0.5	16.16	14.1	110.1	134	103.53	1.29
Jul-11	ŝ	1.5	17.10	18.4	111.1	124	106.72	1.16
Jul-11	С	2.5	16.35	19.7	108.3	110	107.46	1.02
Jul-11	с	3.5	16.46	20.7	108.2	101	109.70	0.92
Jul-11	ŝ	4.5	15.77	21.0	106.2	96	108.40	0.89
Jul-11	m	5.5	16.03	21.6	106.1	06	110.03	0.82

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Table 14. Cont'd.

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DATE	SITE	DEPTH (m)	DEPTH (m) TEMP (°C)	SALINITY (‰)	TGP (%)	DO <sub>2</sub> (% sat)	N2 & Ar (% sat)	O <sub>2</sub> :N <sub>2</sub>
Jul-15	) r	0.5	21.05	13.6	111.9	124	110.37	1.12
Jul-15	Ś	1.5	20.21	16.5	113.2	125	111.68	1.12
Jul-15	Ś	2.5	20.30	17.0	112.5	109	114.94	0.95
Jul-15	ς	3.5	18.58	19.3	110.8	100	113.73	0.88
Jul-15	Ś	4.5	17.73	20.2	108.2	101	110.24	0.92
Jul-15	ŝ	5.5	17.60	20.5	108.5	98	111.52	0.88
Jul-16	Ś	0.5	19.13	15.7	114.0	144	106.57	1.35
Jul-16	Ś	1.5	19.09	15.8	114.7	143	108.04	1.32
Jul-16	ŝ	2.5	19.62	17.2	115.0	132	110.84	1.19
Jul-16	ŝ	3.5	19.54	18.0	114.6	122	112.22	1.09
Jul-16	ŝ	4.5	18.08	18.4	110.9	109	111.21	0.98
Jul-16	ω	5.5	18.20	19.8	109.6	95	112.98	0.84
Jul-17	ŝ	0.5	20.16	15.9	112.7	129	110.24	1.17
Jul-17	m	1.5	20.21	16.5	113.9	127	111.77	1.14
Jul-17	Ś	2.5	20.78	17.4	113.9	121	113.12	1.07
Jul-17	ŝ	3.5	20.00	18.1	113.7	105	116.02	0.90
Jul-17	Ś	4.5	18.76	18.7	110.8	98	114.10	0.86
Jul-17	m	5.5	17.87	19.2	110.2	88	116.12	0.76

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Note: Site 3 was located 250 m east of the BGS thermal discharge point.

DATE	SITE	DEPTH (m)	TEMP (°C)	TEMP (°C) SALINITY (‰)	TGP (%)	DO <sub>2</sub> (% sat)	$N_2$ & Ar (% sat)	$O_2:N_2$
Jul-18	m	0.5	19.77	16.3	110.8	128	106.80	1.20
Jul-18	ſ	1.5	19.78	16.4	111.4	124	108.90	1.14
Jul-18	ς	2.5	20.27	17.7	111.1	120	109.03	1.10
Jul-18	Ś	3.5	18.49	19.0	108.8	105	110.47	0.95
Jul-18	ŝ	4.5	17.39	19.5	106.2	67	109.49	0.89
Jul-18	С	5.5	16.44	20.0	103.6	89	108.13	0.82
A A	r	20	10.47	73 7	110.2	101	107 66	1 1 2
Aug-17	רי רי	C.U A	10.10	73.67	108.4	114	106.62	1 07
Aug-19 ماريم	<u>,</u> 4	ر. ا ۲	18.40	23.5	107.5	102	109.25	0.93
Allo-19	) ( <b>r</b>		17.08	23.7	106.3	93	109.52	0.85
Aug-19	) (n	4.5	16.06	23.8	101.6	82	106.42	0.77
Aug-19	ŝ	5.5	14.90	23.8	102.4	76	108.80	0.70
Aug-20	(1)	0.5	18.93	23.4	111.0	124	106.99	1.16
Aug-20	i m	1.5	18.84	23.6	111.3	118	108.99	1.08
Aug-20	ŝ	2.5	18.73	23.6	110.0	116	107.96	1.07
Aug-20	Ś	3.5	18.59	23.8	107.1	102	108.20	0.94
Aug-20	m	4.5	17.64	23.9	105.3	88	108.94	0.81
Aug-20	С	5.5	16.25	24.1	102.8	88	106.64	0.83
Note: Site 2	3 was loca	Note: Site 3 was located 250 m east of the		BGS thermal discharge point.				

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Table 14. Cont'd.	Cont'd.						
DATE	SITE	DEPTH (m)	TEMP (°C)	TH (m) TEMP (°C) SALINITY (‰)	TGP (%)	DO <sub>2</sub> (% sat)	N2 & Ar (% sat)
Aug-21	Ś	0.5	18.84	23.3	106.9	109	106.21
Aug-21	Ś	1.5	18.39	23.6	107.2	101	108.69
Aug-21	m	2.5	16.84	23.9	102.9	88	106.84
Aug-21	m	3.5	16.43	23.9	101.7	86	105.77
Aug-21	ς	4.5	15.76	24.0	100.1	81	104.39
Aug-21	ŝ	5.5	15.73	23.9	99.5	78	104.79
( ( -	Ċ	د د				( ; ;	
77-guA	Ĵ	C.U	C1.81	0.07	0.601	110	107.01
Aug-22	Ś	1.5	18.00	23.9	108.1	105	108.84
Aug-22	m	2.5	17.91	24.0	105.9	67	107.91
Aug-22	m	3.5	17.05	24.1	103.9	92	106.51
Aug-22	С	4.5	16.32	24.2	103.3	92	106.16
Aug-22	Ś	5.5	16.65	24.1	101.4	91	103.32
, r	-	u C		\ + +	ί ί τ		
07-unr	4	C.U	10.00	14.0	C.CU1	C.601	10/.00
Jun-26	4	1.5	16.29	16.6	105.1	100.9	108.92
Jun-26	4	2.5	14.80	20.6	103.5	85.3	110.24
Jun-26	4	3.5	13.77	22.1	102.2	81.7	109.54

O<sub>2</sub>:N<sub>2</sub>

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1.03 0.93 0.82 0.81 0.78 0.74

> **Note:** Site 3 was located 250 m east of the BGS thermal discharge point. Site 4 was located 1200 m east of the BGS thermal discharge point.

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1.01 0.96 0.90 0.86 0.87 0.88

 $\begin{array}{c} 1.02 \\ 0.93 \\ 0.77 \\ 0.75 \\ 0.71 \end{array}$ 

0.65

108.09 108.03

77.1 69.8

100.6 99.0

22.6 23.0

12.73 12.23

4.5 5.5

4 4

Jun-26 Jun-26

DATE	SITE	DEPTH (m)	TEMP (°C)	EMP (°C) SALINITY (‰)	TGP (%)	$DO_2$ (% sat)	N <sub>2</sub> & Ar (% sat)	O <sub>2</sub> :N <sub>2</sub>
Jul-08	4	0.5	16.10	17.7	106.6	117	102.88	1.14
Jul-08	4	1.5	16.15	20.1	106.7	113	104.57	1.08
Jul-08	4	2.5	14.71	22.0	103.0	91	105.80	0.86
Jul-08	4	3.5	14.13	22.6	103.4	82	107.85	0.76
Jul-08	4	4.5	13.17	22.8	100.7	17	105.96	0.73
Jul-08	4	5.5	12.60	22.7	9.99	68	107.31	0.63
Jul-09	4	0.5	16.94	13.2	108.5	116	106.04	1.09
Jul-09	4	1.5	15.34	19.6	108.7	122	103.54	1.18
Jul-09	4	2.5	15.45	21.0	106.4	106	106.31	1.00
Jul-09	ব	3.5	15.22	21.3	107.4	95	110.76	0.86
Jul-09	4	4.5	15.06	21.5	105.0	92	108.32	0.85
Jul-09	4	5.5	14.86	21.6	105.5	93	108.40	0.86
Jul-10	4	0.5	16.92	16.5	112.6	143	105.09	1.36
Jul-10	4	1.5	16.42	19.3	111.8	110	111.53	0.99
Jul-10	4	2.5	16.08	20.1	107.3	106	107.61	0.98
Jul-10	4	3.5	15.95	20.6	108.8	104	110.07	0.94
Jul-10	4	4.5	15.87	21.0	106.9	102	107.72	0.95
Jul-10	4	5.5	15.18	21.4	105.8	98	107.64	0.91

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DATE	SITE	DEPTH (m)	TEMP (°C)	SALINITY (‰)	TGP (%)	DO <sub>2</sub> (% sat)	$N_2$ & Ar (% sat)	O <sub>2</sub> :N <sub>2</sub>
Jul-11	4	0.5	16.13	14.5	111.9	142	104.72	1.36
Jul-11	4	1.5	17.05	18.3	111.6	119	108.93	1.09
Jul-11	4	2.5	16.45	20.0	108.0	103	109.06	0.94
Jul-11	4	3.5	16.31	20.5	108.2	66	110.31	06.0
Jul-11	4	4.5	15.84	21.1	105.9	94	108.50	0.87
Jul-11	4	5.5	15.27	21.6	105.2	06	109.04	0.83
Jul-15	4	0.5	21.18	13.6	114.7	129	112.15	1.15
Jul-15	4	1.5	19.03	17.8	125.2	129	124.91	1.03
Jul-15	ব	2.5	18.88	18.9	115.4	107	118.34	0.90
Jul-15	4	3.5	18.00	19.6	113.8	92	120.29	0.76
Jul-15	4	4.5	16.59	20.6	107.6	85	113.60	0.75
Jul-15	4	5.5	15.89	20.7	103.0	82	109.44	0.75
Jul-16	4	0.5	20.59	15.7	114.6	133	109.98	1.21
Jul-16	4	1.5	20.48	15.7	118.5	136	114.75	1.19
Jul-16	4	2.5	19.21	17.1	118.8	154	112.10	1.37
Jul-16	4	3.5	18.55	17.6	113.3	127	109.19	1.16
Jul-16	4	4.5	17.87	18.8	108.9	101	111.52	0.91
Jul-16	4	5.5	17.25	19.7	105.9	87	111.03	0.78

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Note: Site 4 was located 1200 m east of the BGS thermal discharge point.

Jul-17 Jul-17	SITE	DEPTH (m)	<del>[</del>	EMP (°C) SALINITY (‰)	TGP (%)	$DO_2$ (% sat)	$N_2$ & Ar (% sat)	O <sub>2</sub> :N <sub>2</sub>
In1-17	4	0.5	20.31	15.6	111.6	133	107.76	1.23
	4	1.5	20.07	15.8	112.5	119	111.82	1.06
Jul-17	4	2.5	18.71	17.4	110.0	112	110.08	1.02
Jul-17	4	3.5	18.48	18.2	109.7	104	111.14	0.94
Jul-17	4	4.5	17.92	18.6	108.0	66	110.40	0.90
Jul-17	4	5.5	17.58	18.8	107.9	94	111.18	0.85
Jul-18	4	0.5	20.01	16.1	110.3	128	106.12	1.21
Jul-18	4	1.5	19.74	16.4	111.7	131	107.03	1.22
Jul-18	4	2.5	17.98	18.6	109.5	101	111.32	0.91
Jul-18	4	3.5	16.70	19.4	104.9	83	110.28	0.75
Jul-18	4	4.5	16.72	19.5	104.0	86	108.18	0.79
Jul-18	4	5.5	16.50	19.6	103.9	86	108.02	0.80
Aug-19	4	0.5	20.15	23.1	110.8	119	108.39	1.10
Aug-19	4	1.5	18.99	23.3	112.2	133	106.21	1.25
Aug-19	4	2.5	18.05	23.4	113.1	129	108.72	1.19
Aug-19	4	3.5	16.97	23.5	111.0	133	104.76	1.27
Aug-19	4	4.5	16.54	23.5	111.8	135	104.83	1.29
Aug-19	4	5.5	16.32	23.5	108.0	126	103.43	1.22

Note: Site 4 was located 1200 m east of the BGS thermal discharge point.

Table 14. Cont'd.

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DATE	SITE	DEPTH (m)	TEMP (°C)	SITE DEPTH (m) TEMP (°C) SALINITY (‰)	TGP (%)	DO <sub>2</sub> (% sat)	$N_2$ & Ar (% sat)	O <sub>2</sub> :N <sub>2</sub>
Aug-20	4	0.5	19.06	23.3	110.9	126	106.81	1.18
Aug-20	4	1.5	18.49	23.4	113.0	131	108.19	1.21
Aug-20	4	2.5	17.60	23.6	109.9	111	109.34	1.02
Aug-20	4	3.5	17.23	23.6	110.0	111	109.75	1.01
Aug-20	4	4.5	16.79	23.7	106.4	106	106.14	1.00
Aug-20	4	5.5	16.04	23.8	104.9	88	109.04	0.81
Aug-21	4	0.5	18.96	23.3	111.4	130	106.33	1.22
Aug-21	4	1.5	18.14	23.6	105.6	85	109.89	0.77
Aug-21	4	2.5	17.12	23.7	105.6	102	106.56	0.96
Aug-21	4	3.5	16.94	23.7	106.0	101	107.06	0.94
Aug-21	4	4.5	16.67	23.7	103.9	100	104.38	0.96
Aug-21	4	5.5	16.16	23.8	103.7	96	105.81	0.91
Aug-22	4	0.5	18.73	23.3	110.9	121	107.58	1.12
Aug-22	4	1.5	17.96	23.5	110.9	102	113.04	06.0
Aug-22	4	2.5	17.29	23.8	108.9	112	107.84	1.04
Aug-22	4	3.5	16.94	23.8	104.8	93	107.38	0.87
Aug-22	4	4.5	15.78	24.0	100.8	81	105.15	0.77
Aug-22	4	5.5	14.97	24.0	98.8	76	104.29	0.73

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Note: Site 4 was located 1200 m east of the BGS thermal discharge point.

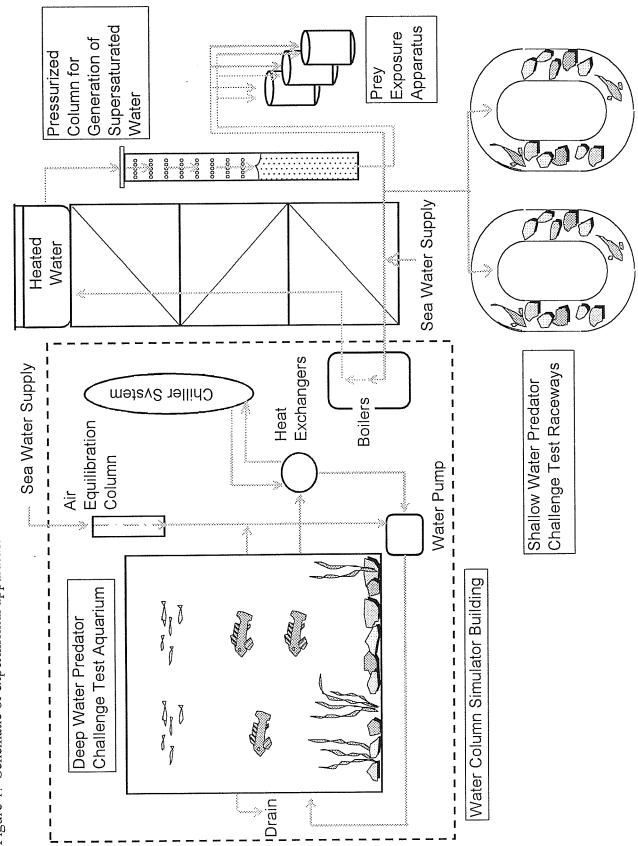
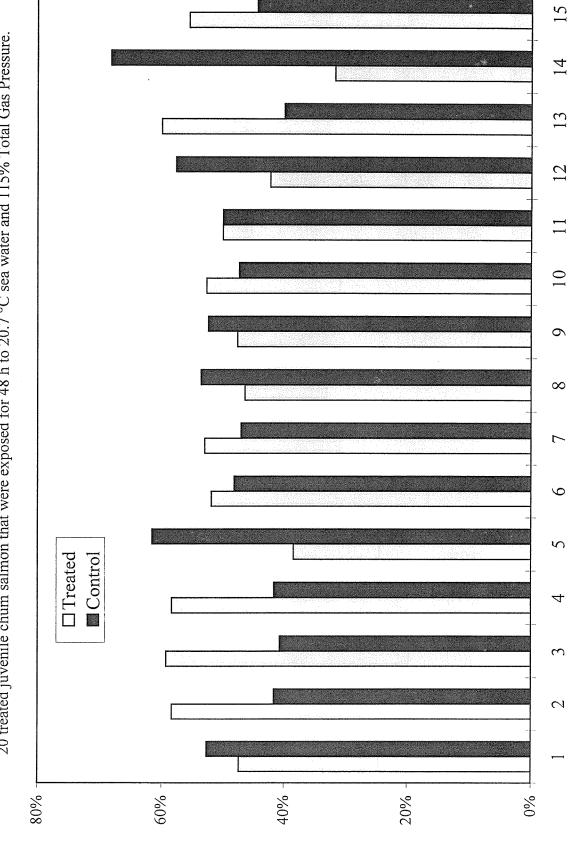


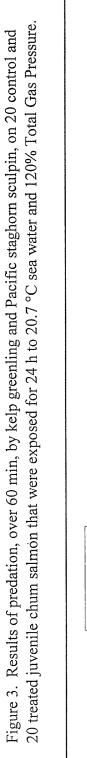
Figure 1. Schematic of experimental apparatus.

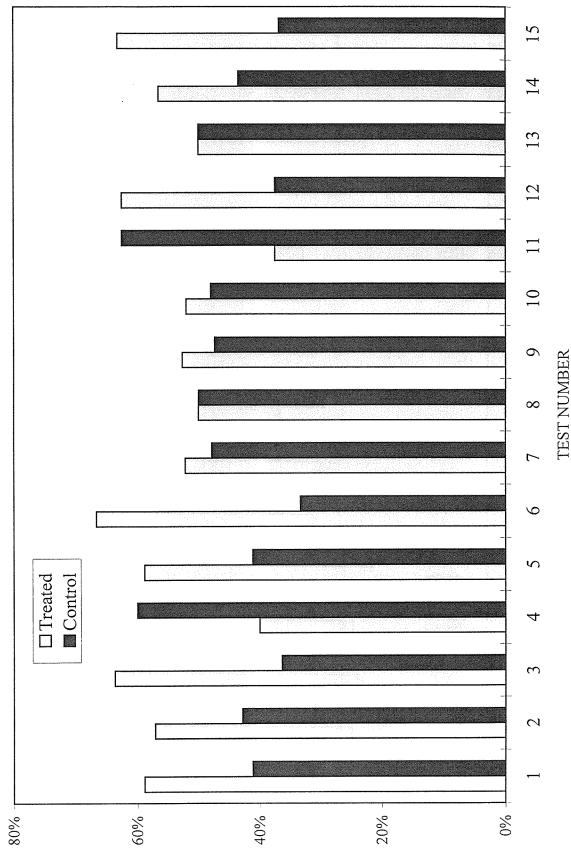


TEST NUMBER

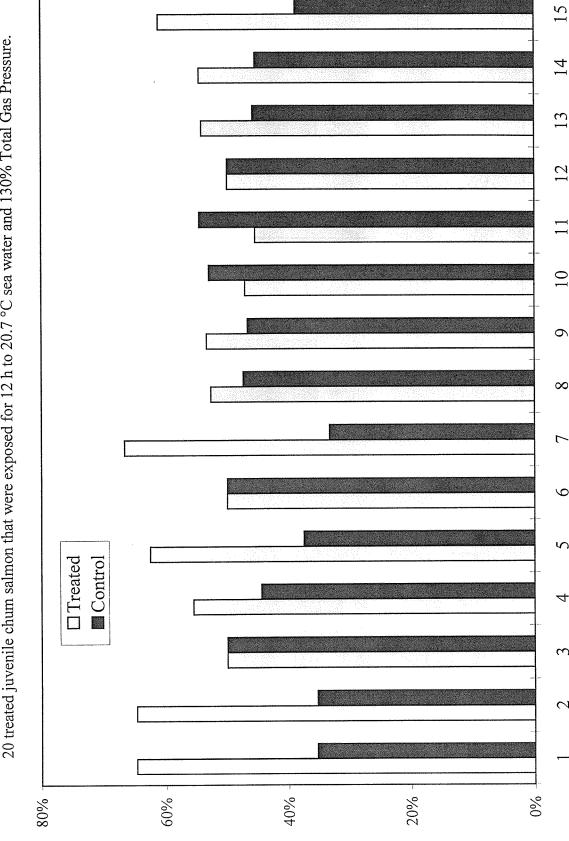


CONSUMPTION (of total number of fish consumed)





CONSUMPTION (of total number of fish consumed)



CONSUMPTION (of total number of fish consumed)

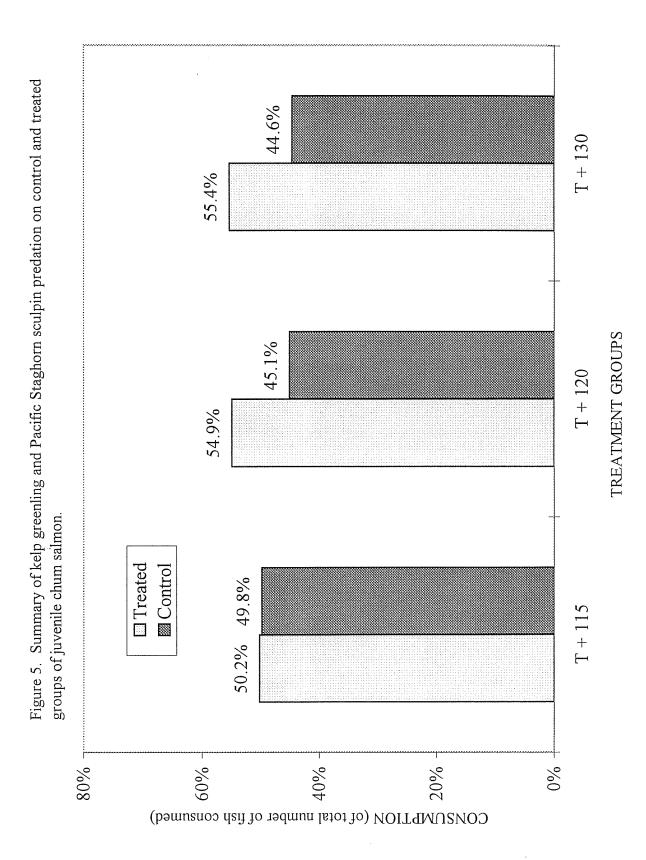
Figure 4. Results of predation, over 60 min, by kelp greenling and Pacific staghorn sculpin, on 20 control and 20 treated juvenile chum salmon that were exposed for 12 h to 20.7 °C sea water and 130% Total Gas Pressure.

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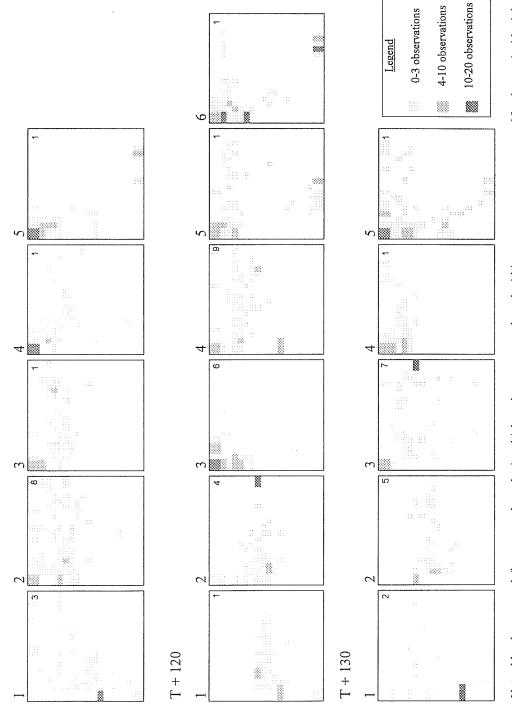
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**TEST NUMBER** 

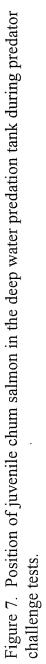




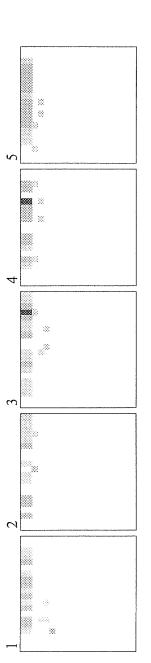
T + 115



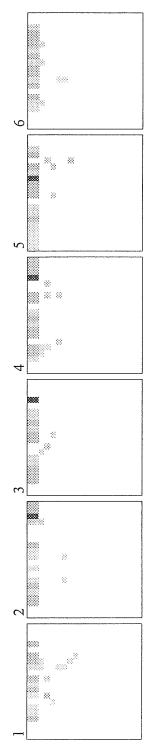
Note: Numbers at top left represent the order in which each test was conducted within any one treatment group. Numbers at inside right represent the order of the tests irrespective of treatment group. Tests ran from June to late September.



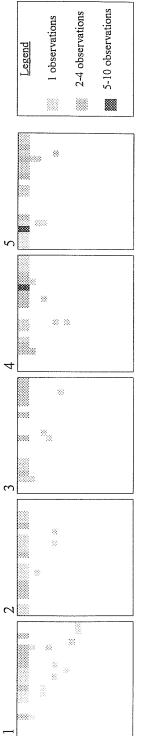
T + 115



T + 120



T + 130



Note: Numbers at top left represent the order in which each test was conducted within any one treatment group. Tests ran from June to late September.

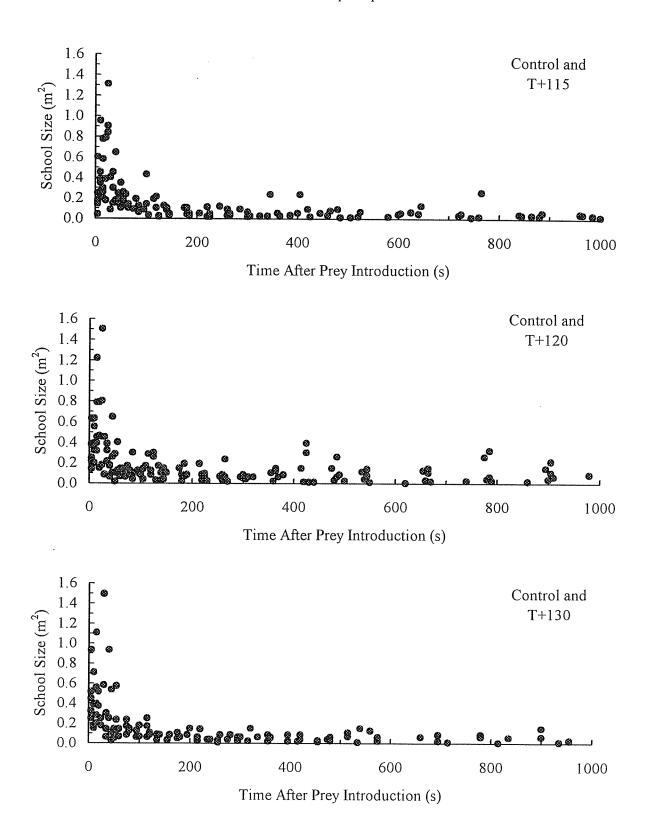
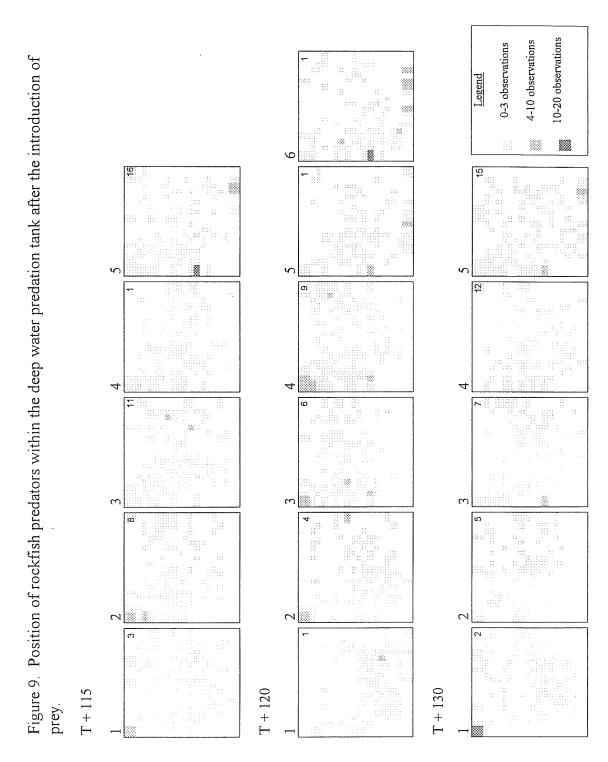


Figure 8. School size as a function of elapsed predation time.





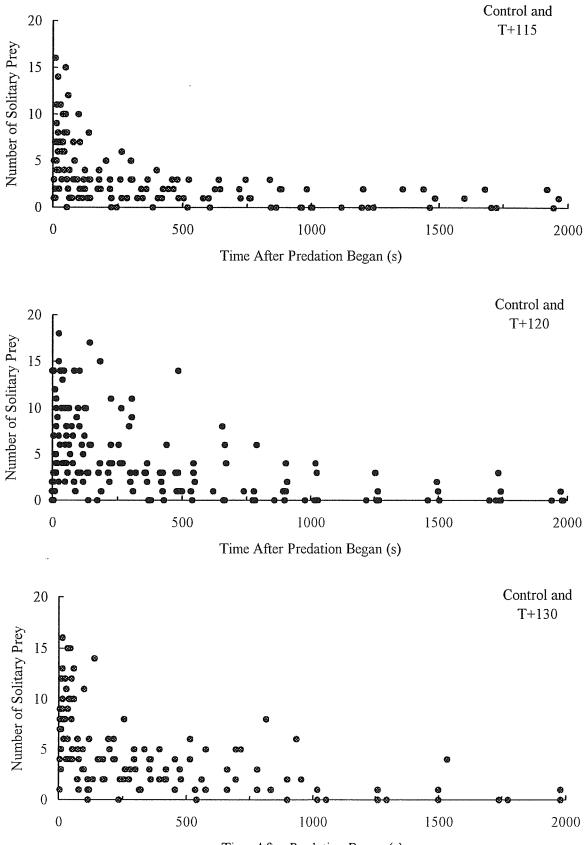


Figure 10. Number of solitary prey as a function of elapsed predation time.

Time After Predation Began (s)

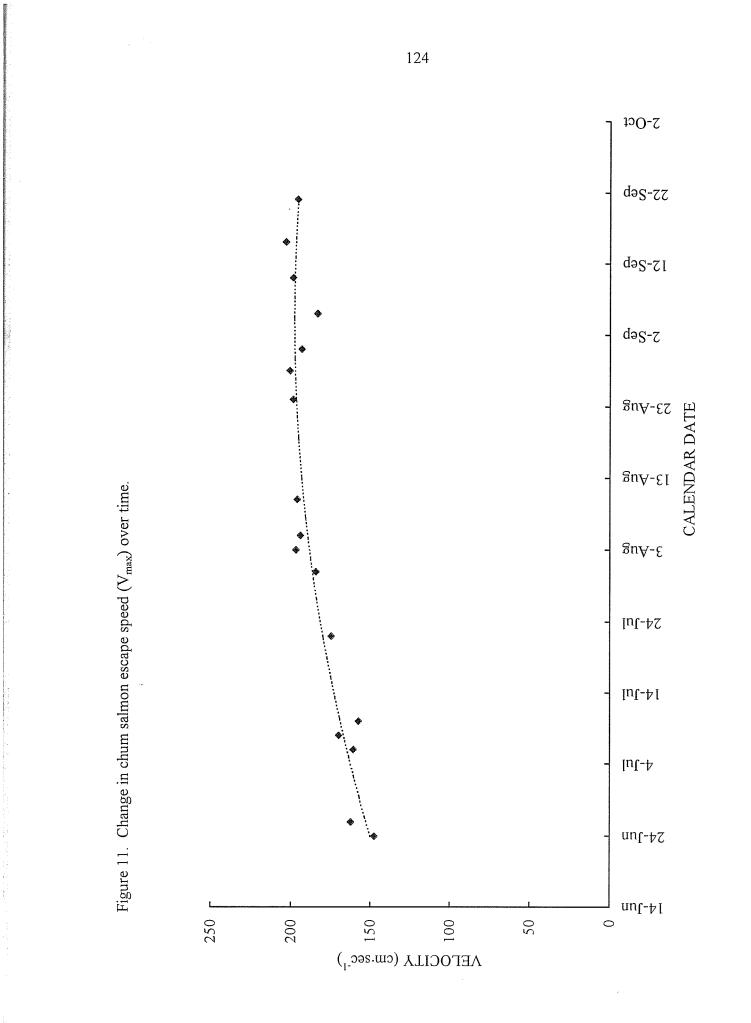
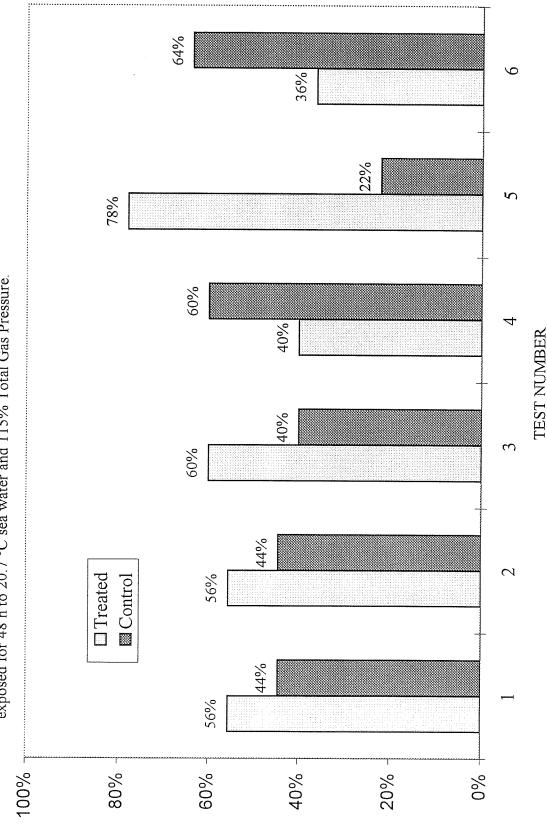
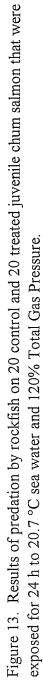
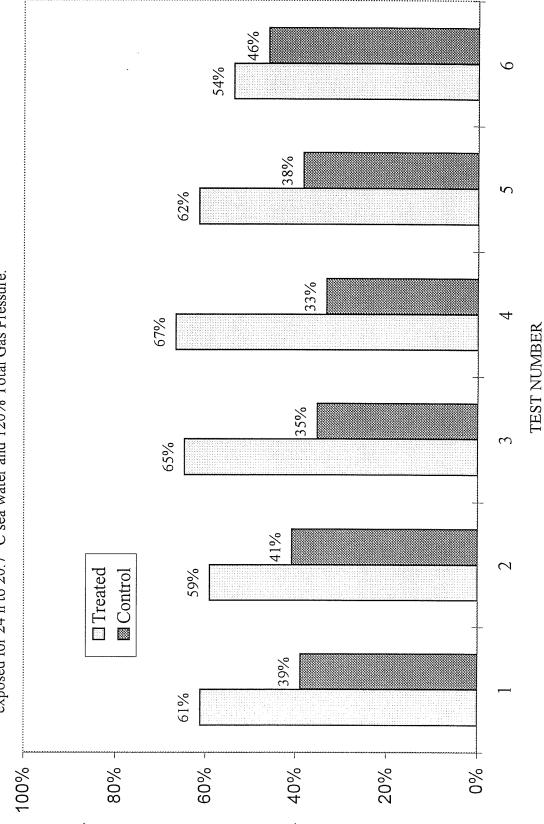


Figure 12. Results of predation by rockfish on 20 control and 20 treated juvenile chum salmon that were exposed for 48 h to 20.7 °C sea water and 115% Total Gas Pressure.

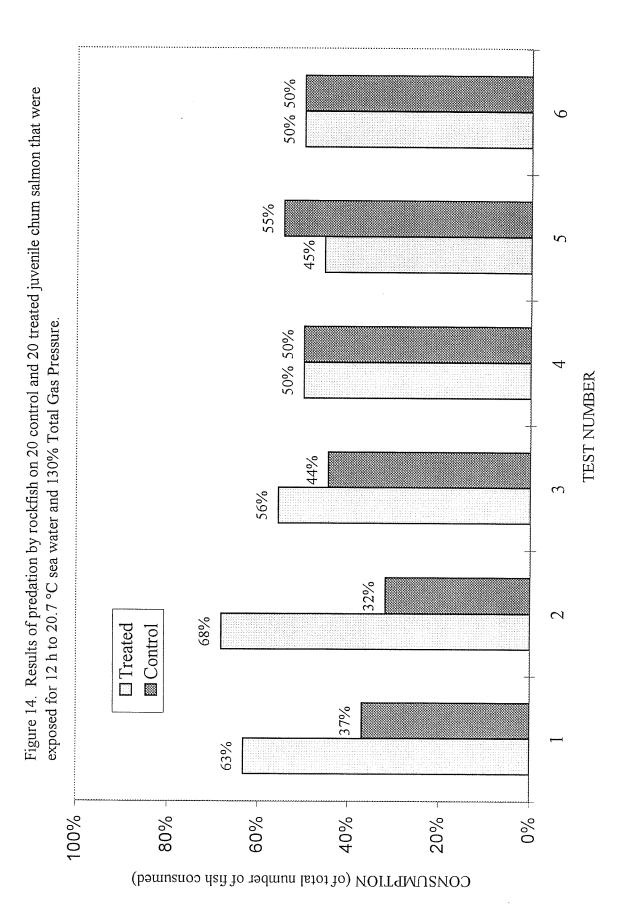


CONSUMPTION (of total number of fish consumed)





CONSUMPTION (of total number of fish consumed)



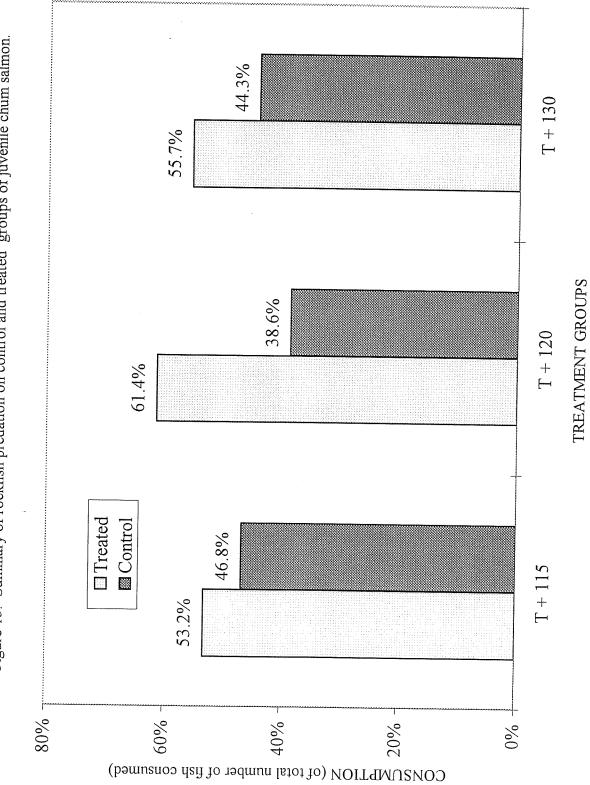


Figure 15. Summary of rockfish predation on control and treated groups of juvenile chum salmon.