An index of respiratory efficiency in the shrimp *Pandalus borealis* (Krøyer) larvae

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AN INDEX OF RESPIRATORY EFFICIENCY IN THE SHRIMP *PANDALUS BOREALIS* (KRØYER) LARVAE

by

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ABSTRACT

Savenkoff, C., L. Saint-Amand, P. Ouellet, and T.T. Packard. 1995. An index of respiratory efficiency in the shrimp *Pandalus borealis* (Krøyer) larvae. Can. Tech. Rep. Fish. Aquat. Sci. 2072: vi+26 p.

Respiration (physiological and enzymatic potential), growth (dry-weight [DW], body and carapace lengths), and protein content were measured on the larval stages of the northern shrimp *Pandalus borealis*. The relationship between oxygen consumption and growth through the larval development was examined under controlled laboratory conditions of temperature and food mixture. Body and carapace lengths, as well as respiration (R) increased as a linear function of time (days) during larval development, whereas biomass, measured as dry-weight and protein content, and enzymatic potential respiration (ETSA) are best described as exponential functions of time during the experiment. There was a decreasing trend in the weight-specific respiration rate (dry-weight-based, Q_{O_2}) during development from hatching to the last larval instar. We estimate that the zoeae of *P. borealis* require a minimum of 2.95 to 0.44 J mg DW^{-1} d^{-1} from the zoeae I stage to the megalopa stage. We propose that the R/ETSA ratio could be used as a quantitative index of the sensitivity of shrimp larvae to environmental stress. The first larval instar would be the most vulnerable, since respiration is close to the respiratory capacity. With growth, and the decrease of the R/ETSA ratio, the later larval instars had a higher potential to generate energy and to respond to environmental stress.

RÉSUMÉ

Savenkoff, C., L. Saint-Amand, P. Ouellet, and T.T. Packard. 1995. An index of respiratory efficiency in the shrimp *Pandalus borealis* (Krøyer) larvae. Can. Tech. Rep. Fish. Aquat. Sci. 2072: vi+26 p.

Des larves de la crevette nordique *Pandalus borealis* ont été gardées sous conditions contrôlées au laboratoire de l'émergence jusqu'au premier stade juvénile (stade mégalopa). La respiration (physiologique et enzymatique potentielle) et différents paramètres caractérisant le développement (longueur de la carapace, longueur totale, poids sec, contenu en protéine) ont été mesurés sur les différents stades larvaires. La longueur de carapace, la longueur totale et la respiration physiologique (R) ont augmenté de façon linéaire au cours du développement larvaire. Le poids sec, le contenu en protéine et la respiration enzymatique potentielle (activité ETS ou AETS) ont augmenté de façon exponentielle pendant la durée de l'expérience. L'activité respiratoire ramenée au poids sec (Q_{O_2}) était maximale immédiatement après l'éclosion puis diminuait pour atteindre un minimum en fin de développement larvaire. Nous avons estimé que les zoés de *P. borealis* avaient un besoin énergétique minimal de 2,95 à 0,44 J mg^{-1} de poids sec par jour depuis le zoé stade I au stade megalopa. Nous proposons l'utilisation du rapport R/AETS comme un indice quantitatif de la sensibilité des larves face à un stress environnemental. Nos résultats montrent que les premiers stades larvaires seront les plus vulnérables du fait que la respiration est proche de la capacité maximale respiratoire. Avec la croissance et la diminution du rapport R/AETS, les derniers stades larvaires ont le plus fort potentiel pour générer de l'énergie et ainsi répondre à des stress environnementaux.

PREFACE

In this report, we established baseline knowledge of the respiratory activity on healthy larval shrimp as the pilot study for a project aimed at determining the effect of mercury on the respiratory activity of the shrimp *Pandalus borealis* in 1994-1996. This project will use the results to investigate a potential toxicity test for future toxicity assessment and monitoring.

This work is a contribution to the program "Investigation of respiratory enzymes as sensitive indicators of mercury contamination in the shrimp *Pandalus borealis* larvae" of the Maurice Lamontagne Institute. This work is included in the environmental Canada's Green Plan. The present study was one of many different projects involved in providing scientific assessments of the presence of toxic chemicals in aquatic ecosystems and their effects on fish, fish habitat, and fisheries resources and was supported by grants from Fisheries and Oceans Canada and the Canadian Green Plan Program.

INTRODUCTION

The northern shrimp *Pandalus borealis* (Krøyer) has a discontinuous circumboreal distribution and is an important commercial species for many northern countries. In the Gulf of St. Lawrence (Canada), commercial exploitation of the shrimp stocks began in 1965 and the landed volume reached about 15,000 metric tons in 1990 (Savard and Boudreau 1993). The abundance of the shrimp stocks is influenced by many biological and abiotic factors (Shumway *et al.* 1985). Among the control mechanisms for stock abundance, fluctuation in the mortality rates of the zoeal stages in response to diverse environmental conditions could affect the recruitment level to the adult stocks. In this context, knowledge of the metabolic characteristics of the larval cycle is essential. Moreover, the measurement of physiological responses in individual organisms should form an important component of any environmental or toxicological monitoring program (Widdows and Donkin 1991). To characterize the physiological responses and the physiological state of organisms, measurements of respiratory activity have been found valuable (Widdows 1985; Widdows *et al.* 1990). Respiratory activity is sensitive to environmental stress because metabolism responds quickly to environmental conditions (Wieser and Zeck 1976). Because respiratory enzymes are the chemical basis of metabolism, they can serve as indicators of metabolism.

Oxygen consumption by respiration is mostly due to oxidative phosphorylation driven by the respiratory electron transfer system (ETS) that is embedded in the lipoprotein inner membrane of eukaryotic mitochondria. The measurement of the ETS activity (ETSA assay) provides a method for determining the potential respiration of the respiratory systems (Packard 1985). The ratio of respiration/ETS activity (R/ETSA) represents an index of the respiratory efficiency. Up to now, the R/ETSA ratio was used to convert the ETS activity measurements to physiological respiration. The ratio has been studied in many animal, plant and bacterial species (Packard 1985 and references herein).

Little information exists about *Pandalus borealis* shrimp, and especially shrimp larval metabolism or the relation between its metabolism and its ecology (Seiring and Hopkins 1985; Shumway *et al.* 1985). *P. borealis* exhibits hermaphroditism, smaller specimens are all males and large ones all females. Females carry fertilized eggs until the hatching of the larvae. Released larvae are planktonic and shift to largely benthic life after metamorphosis (Saotome and Ikeda 1990). In this study, we were interested in determining the links between respiration parameters (real physiological and potential respiration), protein biomass, and morphological characteristics through larval development of the shrimp *P. borealis*. We are proposing the use of the ratio, respiration/ETS activity (R/ETSA), as an index of the respiratory efficiency.

MATERIALS AND METHODS

Adult female shrimp were captured between 200 and 240 m depth in the northwestern Gulf of Saint Lawrence in late April 1993. Healthy egg-bearing female shrimp were selected from the catch and transferred to rearing facilities at Maurice Lamontagne Institute (Mont-Joli, Québec, Canada). Female shrimp were placed in two 80 l flow-through rearing tanks (two females per tank). The rearing tanks were monitored for emergence of the larvae. When between 200 to 400 larvae were released in each tank (thereafter called cohort A for tank A and cohort B for tank B),

during a 24 h period, the females were removed and the rearing experiment commenced. The experiment with cohort A lasted 37 d up to the larval stage IV, from 2 May to 6 June 1993. The experiment with cohort B lasted 71 d, from 7 May to 17 July 1993, and ended after the observation of the first megalopa (Stage VI; Haynes 1979) shrimp in the tank.

At the beginning of the rearing experiment in each tank, the zoeae stage I and II were fed *ad libitum* on newly-hatched (~ 24 h) *Artemia salina* (300 nauplii d^{-1} per liter; Platinum grade, San Francisco Brand). The nauplii were first enriched in polyunsaturated fatty acid by immersion for 6 h in 100 ppm solution of the BIOCAPSULON (Agent Chem. Lab., Redmond, WA) agent (Sorgeloos and Léger 1992). The rations were close to 110 and 50 *Artemia*/larvae for the cohort A and the cohort B respectively. The later zoeae stages were fed on adult *Artemia* every two days. As the food ration was constant for the duration of the experiment and the number of shrimp larvae decreased, the number of prey available to the remaining shrimp larvae increased and paralleled the increasing metabolic demands of the larvae. There was no evidence of cannibalism and the removal of the dead larvae minimized the possibility of necrophagy and disease in the tanks during the experiment.

LARVAL GROWTH AND STAGE IDENTIFICATION

At hatching and every 2 or 3 days thereafter, a random sample of 10 shrimp larvae ("sample 1") was taken from each tank for age (i.e., developmental identification) and morphometric measurements (5 larvae) and ETS activity assays (5 larvae). Larval stage was determined following the description presented by Haynes (1979). Larval carapace length (posterior edge of orbit to mid-dorsal posterior margin of carapace) and total length (tip of rostrum to end of telson) were made on the fresh larvae (n = 5) with an optical micrometer mounted on a dissecting lens. These five larvae were then preserved in liquid nitrogen for the ETS activity analysis. The other 5 larvae of "sample 1" were individually lyophilized with a Labconco freeze dryer 8 at 8 µm Hg vacuum for 45 h for the first three stages and 70 h for the last stages to make sure that a constant dry weight was attained. The water content was determined by the difference between wet and dry weights of samples placed in a dessicator and weighed (± 1 µg) on a CAHN C-31 microbalance.

Independently, at irregular intervals during each larval stage, shrimp larvae were sampled for the respiration measurement ("sample 2").

ETS ACTIVITY MEASUREMENTS

For the potential respiration determinations, we used an enzymatic approach (the ETS activity assay) that measures the capacity of the respiratory chain to transfer electrons to oxygen (Packard *et al.* 1974). The electron transfer system activity (ETSA) assay determines the maximum velocity of electron transfer by the dehydrogenases (NADH-, NADPH- and succinate) in the respiratory system. Throughout this paper ETS refers to electron transfer system (Lehninger *et al.* 1993). The principles and the details of the methodology have been discussed by Christensen and Packard (1979) and Packard (1985). The assay procedure of Packard and Williams (1981) was followed in this study. The ETS activity measurements were made on 3 to 5

larvae from "sample 1" and "sample 2" with several replicates. The reduction reaction was assessed spectrometrically at 490 nm with a Milton Roy Spectronic 3000 Array spectrophotometer. The reference wavelength was fixed at 750 nm in order to correct for the sample absorbance due to turbidity (Packard and Williams 1981). However, the incubation was made directly at 18°C in the spectrophotometer and we recorded the kinetic reaction. The enzymatic assay generates a straight line whose slope was used for our calculation of potential oxygen consumption ($\mu l\ O_2\ h^{-1}\ ind^{-1}$). This kinetic determination of the activity defines more precisely the reaction rate than does end point analysis after 20 minutes of incubation. Furthermore, this assay avoided the underestimation related to excess biomass, because we could stop the reaction at any time. This kinetic record saved time (time of incubation, quenched reaction, and centrifugation) and reagents. The ETS activity was corrected from the incubation temperature (18°C; *in vitro*) to 6°C (temperature of the respiratory measurements) using an Arrhenius activation energy of 66 151 J $mole^{-1}$ (or 15.8 kcal $mole^{-1}$; Packard *et al.* 1975).

RESPIRATION MEASUREMENTS

Throughout this experiment respiration refers to oxygen consumption by intact, live organisms. Respiration was measured with a Clark-type oxygen-electrode (Lee and Tsao 1979). This electrode (Rank brothers Digital Oxygen Electrode System; OXSDIG, model 10) had a resolution of 0.1 % oxygen saturation. The OXSDIG system was linked to a computer for continuous recording of the data. We used the data analysis software, Easyest LX, from Asyst Software Technologies, Inc. The measurement chamber had a capacity of 50 ml and contained a mesh protection system (20 µm) to reduce the stirring effect on the larvae. Accurate respiration measurements necessitated the use of a current stabilizer and a constant temperature water bath (± 0.1°C).

Active larvae (19 for smaller zoeae and 7 for larger zoeae; "sample 2") in good condition were transferred to the reaction vessel kept at 6°C. We used 27 ml of air-saturated sea water in the measurement chamber. The sea water was filtered on 0.2 µm to prevent bacterial respiration during the experiments. Before each measurement the larvae were acclimated for 20 minutes to the experimental conditions. Great care was taken to preserve the same *in situ* environmental conditions throughout the preparatory and measurement phases. Voltage was continuously recorded for 1 hour at intervals of 30 seconds. The results were converted from millivolt per second to $\mu l\ O_2\ l^{-1}\ h^{-1}$, using calibration curves based on a sodium dithionite zero and air-saturated water. The electrode was calibrated prior to and after the measurement. The oxygen in the air-saturated water was determined by the Winkler method as described by Aminot and Chaussepied (1983). We used the automated dissolved oxygen titration system developed by Jones *et al.* (1992). The salinity was determined by the measurement of the conductivity with an Autosal Guildline model 8400. After the respiration experiment, the larvae were preserved in liquid nitrogen until the analyses of the ETS activity and total protein.

Because ETS activity is easier and faster to measure than respiration, we have more ETSA data. As respiration was measured at a lower sampling frequency than dry weight or ETS activity, "estimated respiration" values corresponding to dry weight data were generated from the equation describing measured respiration versus time (day) of development (see Results: Oxygen consumption).

To avoid confusion, we define the terms that we will use through out this paper.
Respiration: Respiratory rate of the live organisms measured with a polarographic oxygen-electrode. This is a physiological process.
ETS activity (ETSA): Enzymatic respiratory activity in a cell-free extract (a homogenate prepared from the shrimp larvae). This is a biochemical reaction rate, theoretically the potential respiration, i.e., the maximum oxygen consuming capacity of the larvae.
Estimated respiration: Respiration calculated from the regression equation of measured respiration with the time (day) of development (see Results: Oxygen consumption).

PROTEIN ANALYSIS

Protein measurements were made according to the method of Lowry *et al.* (1951) using bovine serum albumin as a standard. Analyses were made on the larvae used in the respiration measurements ("sample 2"). The larvae (3 to 5) were homogenized together for 2 min at room temperature in 2 to 4 ml NaOH (1N). The homogenate was diluted (2 to 10 times) in NaOH and a 0.5 ml aliquot used in the assay procedure. The cellular debris was removed by centrifugation (2 min) with an Eppendorf centrifuge (model 5402) at 2000 RPM at room temperature. Colorimetric measurements were made at 750 nm with a Milton Roy Spectronic 3000 Array Spectrophotometer. Duplicate protein measurements were made on each homogenate.

RESULTS

LARVAL MOULT FREQUENCY AND GROWTH

A total of 209 shrimp larvae were enumerated in cohort A. Despite the lack of temperature and salinity control, the mean tank temperature and salinity were relatively stable throughout the experiment. The mean tank temperature and salinity were 5.5 ± 0.7 °C and 26.7 ± 1.2 for the 37 days of the experiment. Development was synchronous among the larvae of cohort A and three moults were observed, at intervals of about 10 days, during the period: all zoeae I larvae had moulted to zoeae II after 9 days, to zoeae III after 19 days and to zoeae IV larvae after 33 days (Fig. 1). Survival from hatching to the zoeae IV stage was 80%.

A total of 450 larvae comprised cohort B. They were studied for 71 days. During this time the mean temperature and salinity were 6.0 ± 1.5 °C and 26.4 ± 1.3 respectively. Shrimp larval development was monitored to the megalopa stage (stage VI; Haynes 1979). Development up to zoeae III in cohort B followed the same pattern as that observed in cohort A (Fig. 1). The larvae moulted synchronously at intervals of about 10 days: all zoeae I larvae had moulted to zoeae II after 9 days and to zoeae III after 19 days. Development to zoeae IV took longer (about 18 days) but all zoeae IV larvae had moulted to zoeae V after 47 days, i.e., a 10 day interval. The first megalopa was observed 20 days later, two days before the end of the experiment (Fig. 1). Survival from hatching to the megalopa was 86%.

The morphological and physiological characteristics of the shrimp larvae were similar between the cohorts during the same period of larval growth (Tables 1 and 2; Fig. 2). Both total and

carapace lengths in cohorts A and B increased as a linear function of time (Fig. 2, Tables 1 and 2). Wet and dry weight increased during larval development and were highly correlated ($r = 0.99$, $n = 125$, $p < 0.001$). Water content of shrimp larvae was relatively constant (84%) from hatching to the zoeae IV stage, and decreased (80% and 81%) in the later stages (Table 1).

In cohort B, dry weight and protein content increased exponentially during development (Fig. 2). The rates of increase (slope of the regression) in larval dry weight and protein content were low (0.02 mg d^{-1} for these two parameters respectively) from hatching to the zoeae III stage, but increased to 0.11 mg d^{-1} for dry weight and 0.06 mg d^{-1} for protein content from the zoeae IV to the megalopa stages (Fig. 2). Shrimp larval growth (biomass) through time was described (at the mean rearing temperatures) by:

$$\ln(y) = a\,t + b \qquad (1)$$

where y is the larval biomass (dry weight or protein content), t is time since hatching (days), and a and b are the adjusted parameters (Dawirs 1983; Table 3). Since b is the intercept with the logarithmic (y) axis in equation (1), the expression e^b represents an estimate of the initial (day 0) larval biomass. The initial larval biomass were 0.19 and 0.21 mg ind^{-1}, respectively for dry weight and protein content (cohort B). The fact that protein content was superior to dry weight, was due only to the statistical method we used. However, this result showed that the main body composition is protein. The body composition data of *P. borealis* and other decapod larvae suggested low food reserves (depleted of triacylglycerol reserves at hatching) in their bodies, indicating the priority of development and growth over the accumulation of reserves early in development (Anger 1986; Dawirs 1987; Saotome and Ikeda 1990; Ouellet *et al.* 1992). Total protein content represented 21% of shrimp larvae wet-weight in the zoeae I stage then decreased to a relatively stable value of ~12% of the wet weight from zoeae III stage to megalopa stage.

OXYGEN CONSUMPTION

Respiration increased progressively from 1.24 µl O_2 h^{-1} ind^{-1} for the zoeae I stage to 4.08 µl O_2 h^{-1} ind^{-1} for megalopa stage (Table 1). The ETS activity increased more quickly (Table 1 and Fig. 2) and was highly correlated with respiration during larval growth (Table 2, Fig. 3). The changes between the respiration and the ETS activity throughout the experiment, were illustrated by a drop in the R/ETSA ratio from 0.66 (zoeae I stage) to 0.21 (megalopa stage). Respiration, ETS activity, growth (dry weight, body and carapace lengths), and protein content, were highly correlated during the experiment (Table 2). During the larval growth, respiration increased as a linear function of time, while ETS activity, protein and dry weight increased exponentially with time (Table 2). The relationships between respiration, ETS activity and dry weight during larval growth were:

$$ETSA = 4.35\,DW + 1.13 \qquad (r = 0.97, n = 26, p < 0.001) \qquad (2)$$

$$R_{est} = 0.77\,DW + 1.09 \qquad (r = 0.94, n = 26, p < 0.001) \qquad (3)$$

where ETSA is ETS activity (µl O_2 ind^{-1} h^{-1}), DW is dry weight per individual (mg), and R_{est} is estimated respiration (µl O_2 ind^{-1} h^{-1}). Data on dry weight and oxygen consumption are usually fitted to logarithmic functions (Dawirs 1983). Accordingly, we performed the regression analysis

on the log-transformed data as well, but the correlation coefficients were relatively similar to those obtained for untransformed data.

$$\ln(ETSA) = 0.78 \ln(DW) + 1.77 \quad (r = 0.98, n = 26, p < 0.001) \quad (4)$$

$$\ln(R_{est}) = 0.53 \ln(DW) + 0.70 \quad (r = 0.99, n = 26, p < 0.001) \quad (5)$$

ETS as a function of dry weight could be fit equally well by a linear equation (2) or by a log-transformed equation (4), while the relationship between estimated respiration and dry weight is higher with the log-transformed data. The slopes (linear equation (3): 0.77; and logarithmic equation (5): 0.53) of the relations between estimated respiration and dry weight were lower than 1. In general, individual animal biomass increases faster than oxygen consumption, i. e., weight specific respiration rates decrease with increasing biomass.

We determined the distribution of the dry weight-specific oxygen consumption rates (Q_{O_2}) for both the ETS activity and the "estimated respiration" on *P. borealis* larvae of cohort B with respect to the time (days) of development (Fig. 4a). The distribution of the dry weight-specific oxygen consumption rates (Q_{O_2}) for both the ETS activity and the "measured respiration" on *P. borealis* larvae of cohort B with respect to the larval stage is shown in Fig. 4b. The respiration-Q_{O_2} and the ETS activity-Q_{O_2} was high immediately after hatching and then declined with age and stage. The lowest values occurred at stage VI (Fig. 4). The absolute difference between the respiration-Q_{O_2} and the ETS activity-Q_{O_2} remained relatively constant during the experiment.

Based on the respiration-Q_{O_2} measurements, and assuming that the main metabolic substrate is protein (Anger and Nair 1979), we calculated the daily energy expenditure due to respiration using a conversion factor of 19.80 10^{-3} J μl^{-1} O_2 (Crisp 1971; 1 cal = 4.1868 J; Table 3). Accordingly, the zoeae of *P. borealis* require a minimum of 2.95 J [mg DW]$^{-1}$ d^{-1} in the zoeae I stage, but only 0.44 J [mg DW]$^{-1}$ d^{-1} in the megalopa stage (Table 3).

DISCUSSION

LARVAL GROWTH CHARACTERISTICS

Body and carapace length increased as a linear function of time, but dry weight and protein content increased exponentially during the development of shrimp larvae (Fig.2). These patterns were consistent with other studies of decapod crustacean larvae where mean individual dry weight showed exponential growth with specific rates (slope) between 0.01 and 0.13 (Logan and Epifanio 1978; Stephenson and Knight 1980; Dawirs 1980, 1983). Moult stage duration, i.e., moult frequency, and stage specific larval length were also comparable to previous data on *P. borealis* (Haynes 1979). Hence, we conclude that the rearing conditions were adequate for the complete and normal development of the shrimp larval stages.

The weight-specific respiration rates (Fig. 4) were higher than those found in the literature for *P. borealis* larvae (Paul and Nunes 1983; Saotome and Ikeda 1990), but decreased during development from the first to the last larval instar as shown by Anger *et al.* (1989). The minimum daily respiratory metabolic requirement of *P. borealis* zoeae I stage was estimated at 1.69 J [mg DW]$^{-1}$ (or 0.28 J ind^{-1}) at 6°C, or about 8 times the estimated joule content (0.035 J ind^{-1}) of 1-

day-old *Artemia salina* nauplii (Paul and Nunes 1983). In our study, the larvae of *P. borealis* required a minimum of 2.95 to 0.44 J [mg DW]$^{-1}$ d^{-1} from stage I to the megalopa stage. Paul and Nunes (1983) showed that the average daily caloric intake of 1-day-old *P. borealis* first zoeae was 6 times their estimated respiratory metabolic requirements at 6°C. We did not measure the feeding rate in our study. Nevertheless, we made sure that each shrimp larva could assimilate sufficient organic material for survival and growth (ration of 50 *Artemia*/larvae in cohort B).

RELATIONSHIPS BETWEEN ETS ACTIVITY AND BIOMASS PARAMETERS

The ETS is composed of proteinaceous material embedded in the inner lipoprotein membrane of the shrimp's mitochondria. Its activity follows the same temporal trend as the protein concentration and the dry weight (note the similar slopes of their regression equations; Table 2). In other words, the ETS activity was highly correlated with these biomass characteristics. Other research has shown ETS activity to be correlated with chlorophyll in water samples (Packard and Williams 1981; Packard 1985; Martinez 1991; Del Giorgio 1992) and with carbon and protein in benthic samples (Relexans and Etcheber 1986). It is, in effect, a good index of living biomass (Packard 1985; Relexans and Etcheber 1986). The fact that the ETS activity does not vary with the physiological state of the cells (Blasco *et al.* 1982; Martinez 1992), facilitates its use as a living biomass indicator.

RESPIRATION/ETS ACTIVITY: AN INDEX OF RESPIRATORY EFFICIENCY

With regard to the relationship between respiration (R) and ETS activity (ETSA), ETS activity reflects the respiratory capacity (*Vmax*). It should exceed the respiratory rate under normal conditions yielding a ratio of respiration/potential respiration (R/ETSA) < 1 (Packard 1985). In our work, the R/ETSA ratio decreased from 0.66 for zoeae I stage to 0.21 for the megalopa stage. The R/ETSA ratio changed from high to low values in bacteria, microalgae cultures, and zooplankton when the organisms grew beyond their exponential phase and advanced into their late log and senescent phases of growth (Kenner and Ahmed 1975; King and Packard 1975; Christensen *et al.* 1980; Packard 1985). However, the change was due to the decreasing respiratory rate and not decreasing ETS activity. The ETS activity remained constant, as one would expect for constitutive enzyme systems. In our study, both respiration and ETS activity increased during the growth, but the respiration increase was proportionally less than the ETS activity increase. This differential increase may give the older organisms a greater ability to manage stress. The R/ETSA ratio is similar to the metabolic scope defined by Lucas (1993; p. 59) as the difference between the maximal respiratory rate and the minimal respiratory rate. The R/ETSA ratio could be defined as a simplification of the metabolic power index, S, defined as the ratio of the difference between the respiratory rate measured and the minimal respiratory rate and the difference of the maximal respiratory rate and minimal respiratory rate (Lucas 1993; p. 59).

Respiration is the process by which all organisms generate energy in the form of ATP, produce "molecular skeletons" for biosynthesis of cellular material, and form reducing equivalents (NADH and NADPH) for the metabolic maintenance of the cell. Cells are normally steady-state systems and to maintain such a steady state, they require a dynamic balance between ATP production and consumption. The basal rate is the resting state which occurs below the

organism's capacity to produce and consume energy. When the eukaryotic cell demands more energy, the mitochondrion increases its ATP production and oxygen consumption. When the cell relaxes, the mitochondrion decreases its production of ATP and oxygen consumption. Thus, a deviation of the R/ETSA ratio from a preestablished norm could serve as an index of larval stress (i.e., a higher than normal R/ETSA ratio would indicate physiological stress). In this context, the results (Figs. 4 and 5) would suggest that the first two larval stages will be the most vulnerable to short term stress. In these two stages, the respiration is close to the respiratory capacity (Fig. 5). There is not much leftover capacity to accomodate stress. With development and growth, and the decrease of the R/ETSA ratio, the later stages will have a higher potential to generate energy in response to environmental stress. Such a capacity should manifest itself in increased survival rates with age. This finding is in agreement with the results of Ouellet *et al.* (1992), who found higher mortality at stages I and II. Saotome and Ikeda (1990) also found that the stage II of *P. borealis* is the most critical larval stage under food limited conditions, because they measured the highest weight-specific respiration rate combined with the low body carbon at stage II. The body composition data of *P. borealis* and other decapod larvae indicate low levels of food reserves in their bodies; development and growth evidently have priority over the accumulation of reserves early in development (Anger 1986; Dawirs 1987; Saotome and Ikeda 1990; Ouellet *et al.* 1992).

CONCLUSION

Much of the past and current research on the causes of recruitment variability in marine populations is built on the hypothesis that recruitment is some function of larval abundance and survival. Our experiments show that the ratio of respiration to potential respiration (R/ETSA) decreases as shrimp larvae mature. This means that a safety margin, the difference between potential respiration and respiration, increases with larval age. This safety margin could impact survival rates and hence recruitment. This ratio could be used as a tool to determine the ability of shrimp larvae to survive environmental variability.

We established baseline knowledge of the respiratory activity on healthy larval shrimp as the pilot study for a project aimed at determining the effect of mercury on the respiratory activity of the shrimp *P. borealis*. Koopman *et al.* (1984), Dutka and Bitton (1986), and Burton and Lanza (1987) have already shown that the respiratory electron transfer system (ETS) can detect the impact of toxic contaminants on fresh water organisms. We are proposing the use of the ratio, respiration/ETS activity (R/ETSA), as an index of the respiratory efficiency and as a quantitative index of the sensibility of shrimp larvae to environmental stress.

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REFERENCES

Aminot A., and M. Chaussepied. 1983. Manuel des analyses chimiques en milieu marin, CNEXO, Brest, 395 p.

Anger, K. 1986. Changes in respiration and biomass of spider crab (*Hyas araneus*) larvae during starvation. Mar. Biol. 90: 261-269.

Anger, K., J. Harms, C. Püschel, and B. Seeger. 1989. Physiological and biochemical changes during the larval development of a brachyuran crab reared under constant conditions in the laboratory. Helgol. Meeresunters 43: 225-244.

Anger, K, and K.C. Nair. 1979. Laboratory experiments on the larval development of *Hyas araneus* (Decapoda, Majidae). Helgol. Wiss Meeresunters 32: 36-54.

Blasco, D., T.T. Packard, and P.C. Garfield. 1982. Size dependence of growth rate, respiratory electron transport system and chemical composition in marine diatoms in the laboratory. J. Phycol. 18: 58-63.

Burton, G.A., Jr., and G.R. Lanza. 1987. Aquatic microbial activity and macrofaunal profiles of an Oklahoma Stream. Wat. Res. 21: 1173-1182.

Christensen, J.P., T.G. Owens, A.H. Devol, and T.T. Packard. 1980. Respiration and physiological state in marine bacteria. Mar. Biol. 55: 267-276.

Christensen, J.P., and T.T. Packard. 1979. Respiratory electron transport activities in phytoplankton and bacteria: comparison of methods. Limnol. Oceanogr. 24: 576-583.

Crisp, D.J. 1971. Energy flow measurements, p 197-279. *In* N.A. Holme, and A.D. McIntyre [eds.]. Methods for the study of marine benthos. International Biological Program Handbook No. 16, Blackwell Scientific Publications, Oxford.

Dawirs, R.R. 1980. Elemental composition (CNH) in larval and crab-1 stages of *Pagurus bernhardus* (Decapoda, Paguridae) and *Carcinus maenas* (Decapoda, Portunidae). Mar. Biol. 57: 17-23.

Dawirs, R.R. 1983. Respiration, energy balance and development during growth and starvation of *Carcinus maenas* L. larvae (Decapoda: Portunidae). J. Exp. Mar. Biol. Ecol. 69: 105-128.

Dawirs, R.R. 1987. Influence of limited starvation period on growth and elemental composition (C, H, N) of *Carcinus maenas* (Decapoda: Portunidae) larvae reared in the laboratory. Mar. Biol. 93: 543-549.

Del Giorgio, P.A. 1992. The relationship between ETS (electron transport system) activity and oxygen consumption in lake plankton: a cross-system calibration. J. Plankton Res. 14: 1723-1741.

Dutka, B.J., and G. Bitton. 1986. Toxicity testing using microorganisms, Vol. II, CRC Press, Inc., Boca Raton, Florida.

Haynes, E. 1979. Description of larvae of the northern shrimp, *Pandalus borealis*, reared *in situ* in Kachemak Bay, Alaska. Fish. Bull. U.S. 77: 157-173.

Ikeda, T. 1970. Relationship between respiration rate and body size in plankton animals as a function of the temperature of habitat. Bull. Fac. Fish Hokkaido Univ. 21: 91-112.

Jones, E.P., F. Zemlyak, and P. Stewart. 1992. Operating manual for the Bedford Institute of Oceanography automated dissolved oxygen titration system. Can. Tech. Rep. Hydrogr. Ocean Sci. 138: 51 p.

Kenner, R.A., and S.I. Ahmed. 1975. Correlation between oxygen utilization and electron transport activity in marine phytoplankton. Mar. Biol. 33: 129-133.

King, F.D., and T.T. Packard. 1975. Respiration and the respiratory electron transport system in marine zooplankton. Limnol. Oceanogr. 20: 849-854.

Koopman, B., G. Bitton, C. Logue, J.M. Bossart, and J.M. Lopez. 1984. Validity of tetrazolium reduction assays for assessing toxic inhibition of filamentous bacteria in activated sludge, p. 147-162. *In* L. Dickson, and B.J. Dutka [eds.]. Toxicity screening procedures using bacterial systems. Marcel Dekker, Inc., New York and Basel.

Lee, Y.H., and G.T. Tsao. 1979. Dissolved oxygen electrodes. Advances in Biochemical Engineering 13: 35-86.

Lehninger, A.L., D.L. Nelson, and M.M. Cox. 1993. Principles of Biochemistry. 2nd edition, Worth Publishers, Inc., New York, 1013 p.

Logan, D.T., and C.E. Epifanio, 1978. A laboratory energy balance for the larvae and juveniles of the american lobster *Homarus americanus*. Mar. Biol. 47: 381-389.

Lowry, D.H., N.J. Rosenberg, A.L. Farr, and R.J. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265-275.

Lucas, A. 1993. Bioénergétique des animaux aquatiques, Masson, Paris, 180 p.

Martinez, R. 1991. Biomass and respiratory ETS activity of microplankton in the Barents Sea, p. 193-200. *In* E. Sakshaug, C.E.E. Hopkins, and N.A. Oritsland [eds.]. Proccedings of the Pro Mare Symposium on Polar Marine Ecology. Trondheim, 12-16 May 1990, Pol. Res. 10.

Martinez, R. 1992. Respiration and respiratory electron transport activity in marine phytoplankton: growth rate dependence and light enhancement. J. Plankton Res. 14: 789-797.

Ouellet, P., C.T. Taggart, and K.T. Frank. 1992. Lipid condition and survival in shrimp (*Pandalus borealis*) larvae. Can. J. Fish Aquat. Sci. 49: 368-378.

Packard, T.T. 1985. Measurement of electron transport activity of microplankton, p 207-261. *In* H. Jannasch, and P.J. Leb. Williams [eds.]. Advances in aquatic microbiology, Vol. 3, Academic Press, New York.

Packard, T.T., A.H. Devol, and F.D. King. 1975. The effect of temperature on the respiratory electron transport system in marine plankton. Deep-Sea Res. 22: 237-249.

Packard, T.T., D. Harmon, and J. Boucher. 1974. Respiratory electron transport activity in plankton from upwelled waters. Tethys 6: 213-222.

Packard, T.T., and P.J. Leb. Williams. 1981. Rates of respiratory oxygen consumption and electron transport in surface seawater from the northwest Atlantic. Oceanol. Acta 4: 351-358.

Paul, A.J., and P. Nunes. 1983. Temperature modification of respiratory metabolism and caloric intake of *Pandalus borealis* (Krøyer) first zoeae. J. Exp. Mar. Biol. Ecol. 66: 163-168.

Relexans, J.C., and H. Etcheber. 1986. Characterization of the particulate organic matter in the Loire Estuary (France) using ETS activity measurements. Org. Geochem. 10: 743-749.

Saotome, K., and T. Ikeda. 1990. Body composition and metabolism of the larvae of the pink shrimp (*Pandalus borealis* Krøyer) raised in the laboratory. Bull. Japan Sea Natl. Fish Res. Inst. 40: 67-77.

Savard, L., and M.-M. Boudreau. 1993. Exploitation de la ressource. Colloque sur la crevette du Golfe du Saint-Laurent, Ministère des Pêches et des Océans, Région du Québec, Montréal, 24-26 février 1993.

Seiring, J.V., and C.C.E. Hopkins. 1985. Analyses of starvation-stress on respiration rates, and body weight and composition in the deep-water prawn *Pandalus borealis* from Balsfjorden, Northern Norway, measured during mid-summer in a laboratory experiment, p. 287-298. *In* J.S. Gray, and M.E. Christiansen [eds.]. Marine Biology of Polar Regions and Effects on Marine Organisms. 18th European Marine Biology Symposium, John Wiley and Sons ltd., Chichester.

Shumway, S.E., H.C. Perkins, D.F. Schick, and A.P. Stickney. 1985. Synopsis of biological data on the pink shrimp, *Pandalus borealis* Krøyer, 1838. NOAA Tech. Rep. NMFS 30, FAO Fisheries Synopsis No 144, US Department of commerce, 56 p.

Sorgeloos, P., and P. Léger. 1992. Improved larviculture outputs of marine fish, shrimp and prawn. J. World Aquac. Soc. 23: 251-264.

Stephenson, M.J., and A.W. Knight. 1980. Growth, respiration and caloric content of larvae of the prawn, *Macrobrachium rosenbergii*. Comp. Biochem. Physiol. 66A: 386-391.

Widdows, J. 1985. Physiological responses to pollution. Mar. Pollut. Bull. 16: 129-134.

Widdows, J., K.A. Burns, N.R. Menon, D.S. Page, and S. Soria. 1990. Measurement of physiological energetics (scope for growth) and chemical contaminants in mussels (*Arca zebra*) transplanted along a contamination gradient in Bermuda. J. Exp. Mar. Biol. Ecol. 138: 99-117.

Widdows, J., and P. Donkin. 1991. Role of physiological energetics in ecotoxicology. Comp. Biochem. Physiol. 100C: 69-75.

Wieser, W., and M. Zech. 1976. Dehydrogenases as tools in the study of marine sediments. Mar. Biol. 36: 113-122.

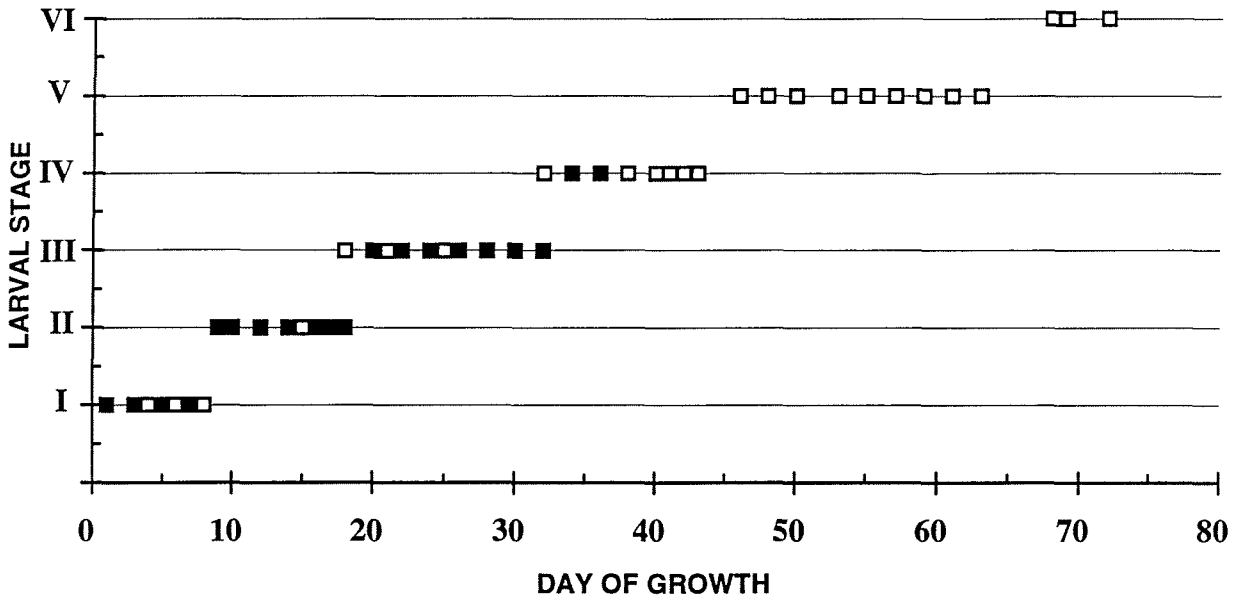

Figure 1. Occurrence of successive larval stages of the shrimp *Pandalus borealis* from the hatching (May 2) to the zoeae IV stage (June 6) in tank A (solid square); and from the hatching (May 7) to the megalopa stage (July 17) in tank B (empty square). Each block represents a day of sampling.

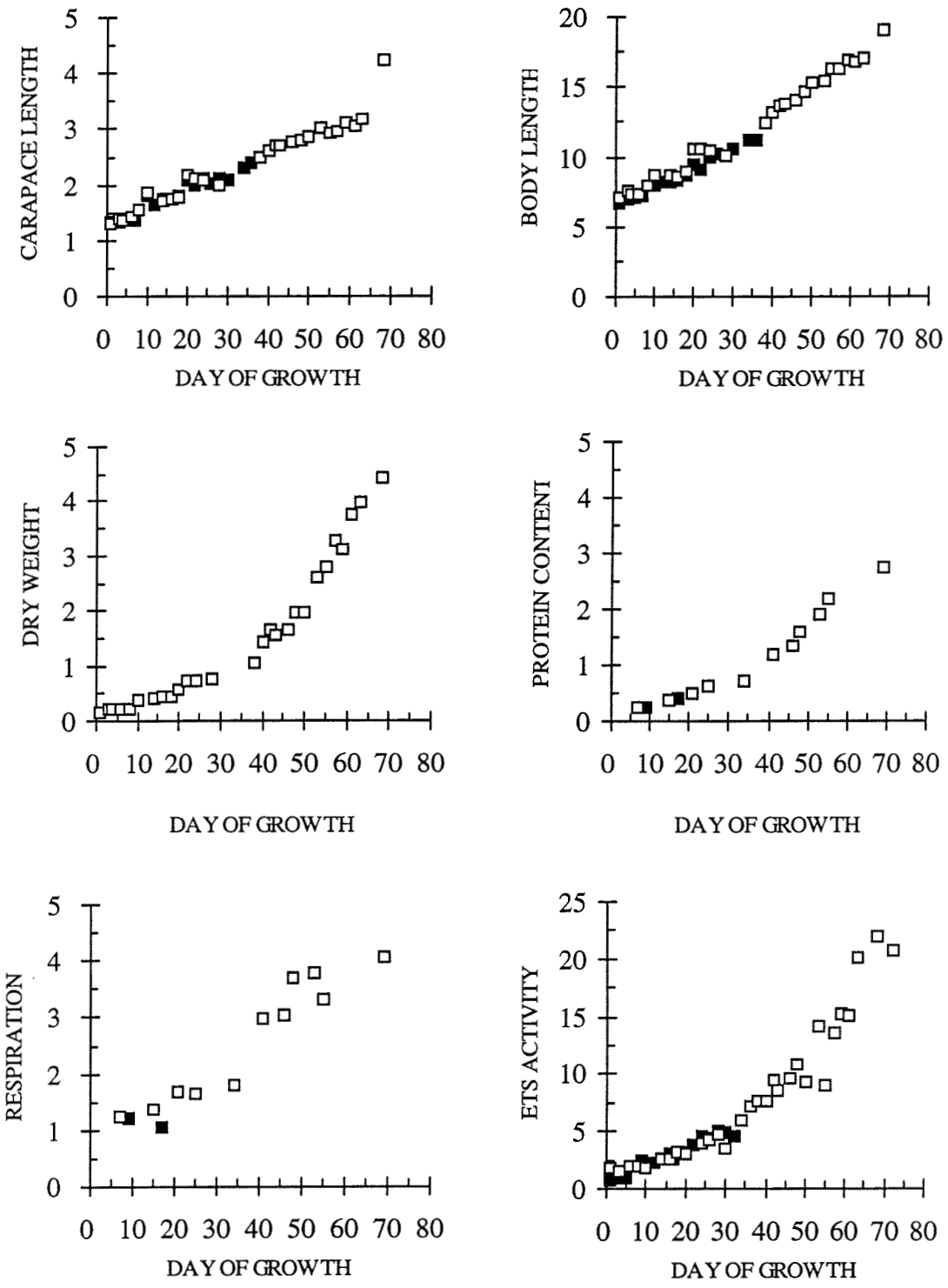

Figure 2. Mean carapace length (mm), mean body length (mm), mean dry weight (mg), mean protein concentration (mg ind^{-1}), respiration (µl O_2 h^{-1} ind^{-1}), and ETS activity (µl O_2 h^{-1} ind^{-1}) of *P. borealis* larvae with respect to the day of growth from the hatching to the zoeae stages IV (tank A, solid square) and VI (tank B, empty square) respectively. Each block represents a day of sampling.

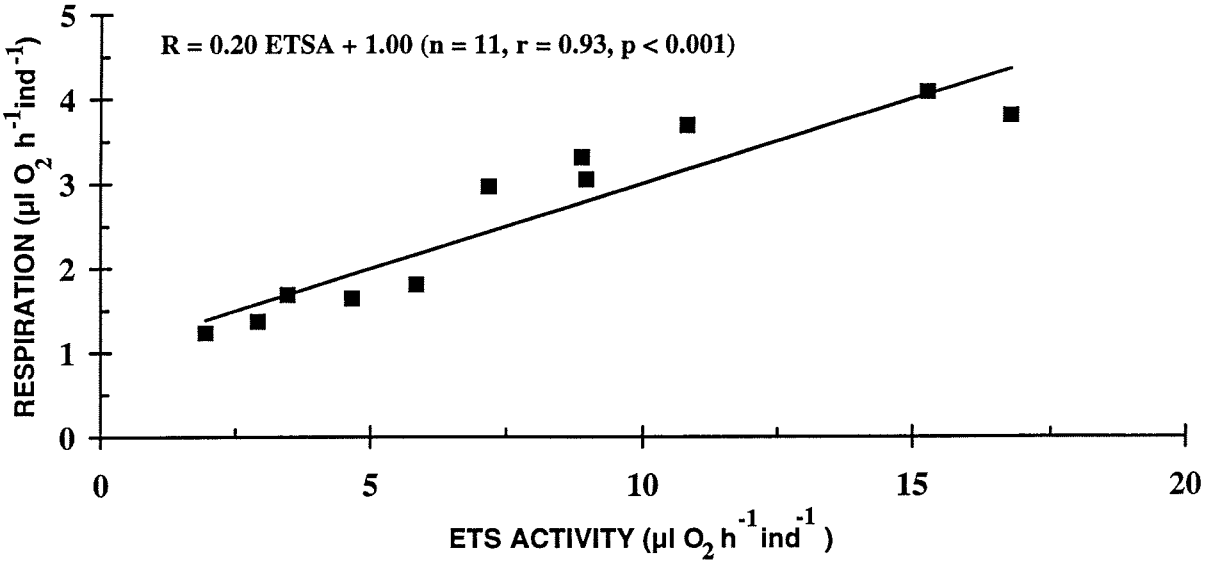

Figure 3. Relationship between ETS activity and respiration from the hatching to the megalopa stage in individuals of *P. borealis* cohort B.

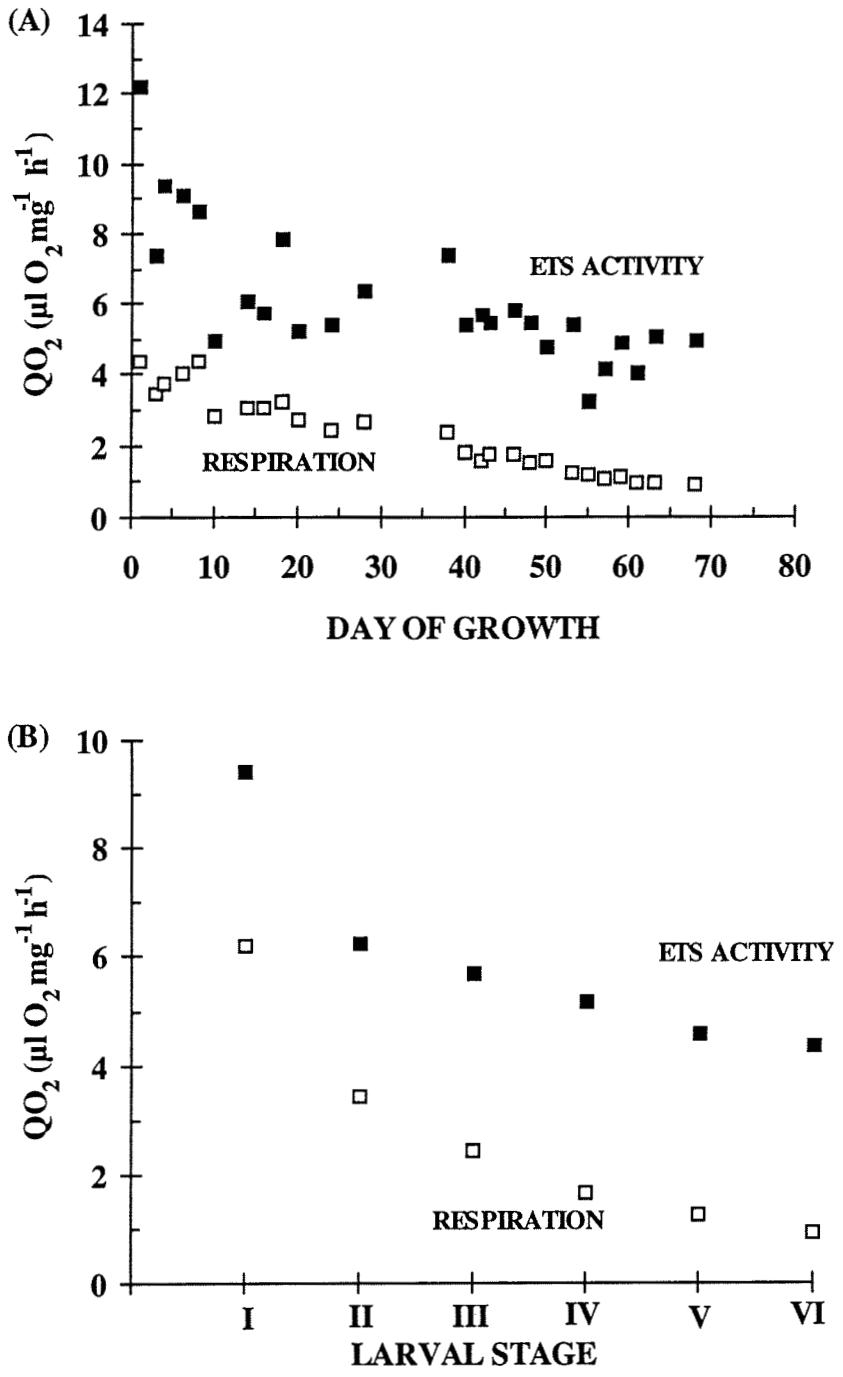

Figure 4. Distribution of the dry weight-specific respiration rates (QO_2) of the *P. borealis* larvae of cohort B with respect to (A) the time (days) of growth for the estimated respiration and ETS activity; and (B) the larval stage for the measured respiration and ETS activity shown in Table 1.

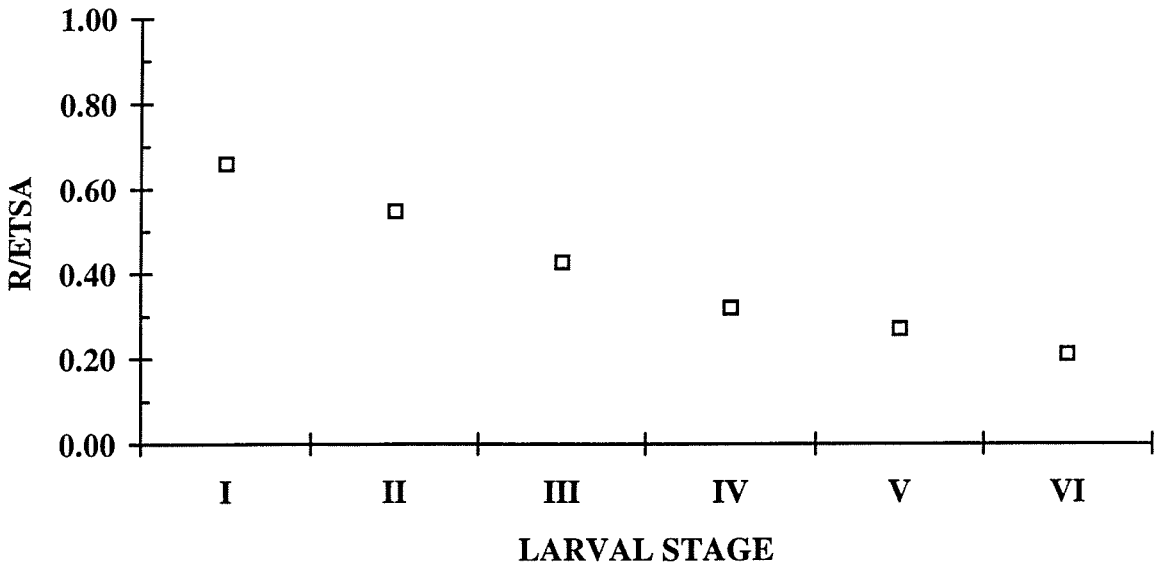

Figure 5. Decrease in the Respiration/ETS activity ratio (R/ETSA) with development (larval stage) of *P. borealis* larvae.

Table 1. Means and standard deviations of total length (TL, mm), carapace length (CL, mm), wet and dry weights (WW and DW, mg), water content (W, %), protein concentration (P, mg ind^{-1}), ETS activity (ETSA, µl O_2 h^{-1} ind^{-1}), and respiration (R, µl O_2 h^{-1} ind^{-1}) for each larval stage of the cohorts A and B (in bold) respectively. In parentheses, we show the number of analyses.

	Larval stage of *Pandalus borealis*					
	I	II	III	IV	V	VI
TL	7.01 ± 0.24 (19)	8.29 ± 0.41 (23)	9.95 ± 0.65 (29)	11.18 ± 0.69 (9)		
	7.29 ± 0.35 (21)	**8.64 ± 0.34 (22)**	**10.34 ± 0.43 (20)**	**13.29 ± 0.94 (22)**	**15.81 ± 1.01 (36)**	**19.00 ± 0.40 (3)**
CL	1.36 ± 0.04 (19)	1.74 ± 0.10 (23)	2.08 ± 0.10 (27)	2.34 ± 0.12 (9)		
	1.37 ± 0.07 (22)	**1.74 ± 0.10 (22)**	**2.09 ± 0.12 (21)**	**2.62 ± 0.20 (22)**	**2.96 ± 0.18 (36)**	**4.21 ± 0.16 (3)**
WW	**1.22 ± 0.30 (22)**	**2.52 ± 0.52 (22)**	**4.18 ± 0.93 (21)**	**8.79 ± 1.85 (22)**	**14.42 ± 4.02 (36)**	**22.18 ± 4.22 (3)**
DW	**0.20 ± 0.05 (21)**	**0.40 ± 0.10 (22)**	**0.69 ± 0.16 (21)**	**1.44 ± 0.35 (22)**	**2.72 ± 0.82 (36)**	**4.42 ± 0.90 (3)**
W	**83.5 ± 1.5 (21)**	**84.1 ± 1.8 (22)**	**83.6 ± 1.0 (21)**	**83.7 ± 1.4 (22)**	**81.2 ± 2.0 (36)**	**80.1 ± 0.6 (3)**
P		0.33 ± 0.09 (4)				
	0.26 ± 0.00 (2)	**0.38 ± 0.01(2)**	**0.56 ± 0.07 (4)**	**0.96 ± 0.27 (4)**	**1.76 ± 0.34 (8)**	**2.70 ± 0.06 (2)**
ETSA	0.85 ± 0.09 (4)	2.56 ± 0.42 (7)	4.34 ± 0.65 (7)			
	1.88 ± 0.17 (6)	**2.50 ± 0.43 (4)**	**3.91 ± 0.60 (8)**	**7.45 ± 1.19 (8)**	**12.48 ± 3.65 (12)**	**19.29 ± 3.55 (3)**
R		1.14 ± 0.08 (2)				
	1.24 (1)	**1.37 (1)**	**1.67 ± 0.02 (2)**	**2.39 ± 0.58 (2)**	**3.46 ± 0.30 (4)**	**4.08 (1)**

Table 2. Relationships between the different parameters from hatching to the larval stage VI of *P. borealis* in cohort B. All the parameters were significantly correlated to each other with a computed probability $p < 0.001$. Time represents days of development; mean body length: mm; mean carapace length: mm; mean dry weight: mg; mean protein: mg ind^{-1}; respiration: $\mu l\ O_2\ h^{-1}\ ind^{-1}$; ETS activity: $\mu l\ O_2\ h^{-1}\ ind^{-1}$; est. respiration ($\mu l\ O_2\ h^{-1}\ ind^{-1}$): respiration calculated from the regression equation with the time (day) of development.

Y	X	correlation coefficient (n)	equation
body length	time	0.99 (27)	Y = 0.17 X + 6.45
carapace length	time	0.97 (27)	Y = 0.03 X + 1.29
dry weight	time	0.94 (27)	Y = 0.06 X - 0.41
		0.99 (27)	ln (Y) = 0.05 X -1.66
protein	time	0.97 (11)	Y = 0.04 X - 0.33
		0.99 (11)	ln (Y) = 0.04 X - 1.54
respiration	time	0.95 (11)	Y = 0.05 X + 0.59
		0.96 (11)	ln (Y) = 0.02 X + 0.03
ETS activity	time	0.94 (26)	Y = 0.26 X - 0.78
		0.99 (26)	ln (Y) = 0.04 X + 0.43
carapace length	body length	0.98 (27)	Y = 0.19 X + 0.06
dry weight	body length	0.99 (26)	ln (Y) = 3.29 ln (X) - 8.11
ETS activity	body length	0.94 (26)	Y = 1.49 X - 10.33
		0.98 (26)	ln (Y) = 2.59 ln(X) - 4.61
dry weight	carapace length	0.93 (27)	Y = 1.70 X - 2.50
ETS activity	carapace length	0.94 (26)	Y = 7.64 X - 10.29
		0.96 (26)	ln (Y) = 2.57 ln(X) - 0.36
ETS activity	dry weight	0.97 (26)	Y = 4.35 X + 1.13
protein	respiration	0.94 (11)	Y = 0.72 X - 0.65
protein	ETS activity	0.89 (10)	Y = 0.15 X + 0.05
respiration	ETS activity	0.94 (10)	Y = 0.20 X + 1.00
est. respiration	body length	0.99 (26)	Y = 0.29 X - 1.23
		0.98 (26)	ln (Y) = 1.75 ln(X) - 3.61
est. respiration	carapace length	0.97 (26)	Y = 1.44 X - 1.14
		0.98 (26)	ln (Y) = 1.75 ln(X) - 0.75

Table 3. Estimated metabolic requirements of *P. borealis* larvae of cohort B. The energy expenditure due to respiration was calculated using a conversion factor of 19.80 10^{-3} J μl^{-1} O_2 for protein metabolism (Crisp 1971; 1 cal = 4.1868 J).

larval stage	oxygen consumption (μl O_2 mg DW^{-1} d^{-1})	mean dry weight (mg)	respiratory energy expenditure (J mg DW^{-1} d^{-1})
I	148.80	0.20	2.95
II	82.20	0.40	1.63
III	58.09	0.69	1.15
IV	39.83	1.44	0.79
V	30.53	2.72	0.60
VI	22.15	4.42	0.44

APPENDIX 1

OBSERVATIONS ON COHORT A OF *PANDALUS BOREALIS* SHRIMP FROM HATCHING (MAY 02, 1993) TO THE ZOEAE IV STAGE (JUNE 06, 1993).

Date	day of growth	larval stage	mean carapace length (mm)	stddev carapace length (mm)	mean body length (mm)	stddev body length (mm)	ETS activity *in vitro* (µl O2/h/ind)	Temperature *in situ* (°C)
02-may-93	1	1					2.55	6.00
02-may-93	1	1	1.33	0.04	6.74	0.28	2.47	6.00
04-may-93	2	1	1.35	0.05	6.99	0.14	2.88	5.60
06-may-93	4	1	1.37	0.02	7.09	0.12	3.12	4.50
08-may-93	6	1	1.38	0.02	7.19	0.21		5.70
10-may-93	8	2					7.69	5.70
11-may-93	9	2	1.79	0.04	7.89	0.17	6.06	5.70
13-may-93	11	2	1.64	0.10	8.18	0.18	7.63	5.60
15-may-93	13	2	1.74	0.11	8.20	0.39	8.59	5.70
17-may-93	15	2	1.75	0.06	8.31	0.29	9.86	5.10
18-may-93	16	2					8.25	4.90
19-may-93	17	2	1.81	0.12	8.72	0.50	9.91	5.20
21-may-93	19	3	2.09	0.12	9.41	0.57	10.28	5.10
23-may-93	21	3	2.00	0.15	9.11	0.51	12.33	4.80
25-may-93	23	3	2.11	0.08	9.96	0.21	14.58	5.00
27-may-93	25	3	2.02	0.08	10.22	0.51	14.12	4.40
29-may-93	27	3	2.10	0.09	10.11	0.51	16.31	4.80
31-may-93	29	3	2.08	0.15	10.56	0.76	15.82	5.60
02-jun-93	31	3					14.95	5.90
04-jun-93	33	4	2.30	0.08	11.13	0.67		6.70
06-jun-93	35	4	2.38	0.15	11.22	0.77		7.30

Date	day of growth	larval stage	ETS activity at 6°C (µl O2/h/ind)	respiration at 6°C (µl O2/h/ind)	mean protein content (µg/ind)	stddev protein content (µg/ind)
02-may-93	1	1	0.79			
02-may-93	1	1	0.76			
04-may-93	2	1	0.89			
06-may-93	4	1	0.96			
08-may-93	6	1				
10-may-93	8	2	2.38	1.22	253.14	1.9
11-may-93	9	2	1.87			
13-may-93	11	2	2.36			
15-may-93	13	2	2.66			
17-may-93	15	2	3.05			
18-may-93	16	2	2.55	1.06	415.81	7.7
19-may-93	17	2	3.06			
21-may-93	19	3	3.18			
23-may-93	21	3	3.81			
25-may-93	23	3	4.51			
27-may-93	25	3	4.36			
29-may-93	27	3	5.04			
31-may-93	29	3	4.89			
02-jun-93	31	3	4.62			
04-jun-93	33	4				
06-jun-93	35	4				

APPENDIX 2

OBSERVATIONS ON COHORT B OF *PANDALUS BOREALIS* SHRIMP FROM HATCHING (MAY 07, 1993) TO THE MEGALOPA STAGE (JULY 17, 1993).

Date	day of growth	larval stage	mean carapace length (mm)	stddev carapace length (mm)	mean body length (mm)	stddev body length (mm)	mean dry weight (mg)	stddev dry weight (mg)
07-may-93	1	1	1.30	0.08	7.06	0.32	0.15	0.05
09-may-93	2	1	1.38	0.06	7.53	0.61	0.21	0.01
10-may-93	3	1	1.37	0.00	7.28	0.15	0.21	0.03
12-may-93	5	1	1.44	0.03	7.30	0.16	0.22	0.04
13-may-93	6	1						
14-may-93	7	1	1.55	0.19	7.93	0.77	0.23	0.07
16-may-93	9	2	1.85	0.04	8.66	0.15	0.38	0.04
20-may-93	13	2	1.70	0.09	8.69	0.21	0.42	0.05
21-may-93	14	2						
22-may-93	15	2	1.75	0.10	8.54	0.57	0.45	0.10
24-may-93	17	3	1.77	0.17	8.97	0.44	0.46	0.07
26-may-93	19	3	2.19	0.09	10.54	0.27	0.59	0.12
27-may-93	20	3						
28-may-93	21	3	2.10	0.09	10.55	0.34	0.73	0.14
30-may-93	23	3	2.10	0.06	10.45	0.21	0.73	0.08
31-may-93	24	3						
01-jun-93	25	3						
03-jun-93	27	3	1.98	0.16	10.01	0.53	0.75	0.19
05-jun-93	29	3						
09-jun-93	33	4						
09-jun-93	33	4						
11-jun-93	35	4						
13-jun-93	37	4	2.48	0.21	12.47	1.10	1.05	0.32
15-jun-93	39	4	2.61	0.22	13.18	1.24	1.42	0.28
16-jun-93	40	4						
17-jun-93	41	4	2.71	0.18	13.70	0.28	1.67	0.34
18-jun-93	42	4	2.70	0.15	13.80	0.53	1.57	0.27
21-jun-93	45	5	2.77	0.25	14.09	0.86	1.67	0.25
21-jun-93	45	5						
23-jun-93	47	5	2.78	0.17	14.72	0.30	1.99	0.40
23-jun-93	47	5						
25-jun-93	49	5	2.86	0.28	15.28	1.10	1.97	0.71
28-jun-93	52	5	3.01	0.17	15.41	0.69	2.62	0.33
28-jun-93	52	5						
30-jun-93	54	5	2.93	0.10	16.22	0.35	2.79	0.31
30-jun-93	54	5						
02-jul-93	56	5	2.96	0.16	16.22	0.69	3.28	0.13
04-jul-93	58	5	3.10	0.10	16.85	0.20	3.13	0.52
06-jul-93	60	5	3.03	0.14	16.74	0.69	3.76	0.56
08-jul-93	62	5	3.17	0.05	17.00	0.40	3.98	0.27
13-jul-93	67	6	4.21	0.16	19.00	0.40	4.42	0.90
14-jul-93	68	6						
17-jul-93	71	6						

Date	day of growth	larval stage	mean water content (%)	stddev water content (%)	ETS activity *in vitro* (µl O2/h/ind)	Temperature *in situ* (°C)	ETS activity at 6°C (µl O2/h/ind)
07-may-93	1	1	83.80	1.85	5.78	4.8	1.79
09-may-93	2	1	82.24	1.94	5.07	5.8	1.57
10-may-93	3	1	83.63	0.94	6.44	6.1	1.99
12-may-93	5	1	83.64	1.12	6.49	5.9	2.01
13-may-93	6	1			6.32	5.8	1.95
14-may-93	7	1	85.86	2.15	6.35	5.7	1.96
16-may-93	9	2	83.77	0.68	6.14	5.9	1.90
20-may-93	13	2	85.05	0.53	8.29	5.1	2.56
21-may-93	14	2			9.46	5.1	2.92
22-may-93	15	2	82.37	0.89	8.43	5.1	2.60
24-may-93	17	3	83.36	1.03	11.64	4.9	3.60
26-may-93	19	3	84.06	1.02	9.96	4.5	3.08
27-may-93	20	3			11.23	4.5	3.47
28-may-93	21	3	83.34	0.50		4.6	
30-may-93	23	3	84.12	1.19	12.77	4.5	3.95
31-may-93	24	3			15.07	7.1	4.66
01-jun-93	25	3			13.79	6	4.26
03-jun-93	27	3	82.88	1.14	15.39	6.3	4.76
05-jun-93	29	3			11.47	6.8	3.54
09-jun-93	33	4			19.80	6.3	6.12
09-jun-93	33	4			18.90	6.1	5.84
11-jun-93	35	4			23.08	6.3	7.13
13-jun-93	37	4	85.00	2.30	24.87	6.3	7.69
15-jun-93	39	4	83.50	0.37	24.70	7.6	7.64
16-jun-93	40	4			23.19	8	7.17
17-jun-93	41	4	83.35	0.79	30.67	8.8	9.48
18-jun-93	42	4	83.72	0.64	27.69	9.3	8.56
21-jun-93	45	5	82.67	0.67	33.43	7.6	10.33
21-jun-93	45	5			28.94	7.6	8.95
23-jun-93	47	5	82.22	1.12	34.99	6.3	10.82
23-jun-93	47	5				6.5	
25-jun-93	49	5	82.37	1.46	30.17	5.9	9.33
28-jun-93	52	5	81.04	0.76	37.07	6.5	11.46
28-jun-93	52	5			54.23	6.5	16.76
30-jun-93	54	5	80.33	1.13	29.72	7.6	9.19
30-jun-93	54	5			28.69	7.7	8.87
02-jul-93	56	5	78.74	4.82	43.92	8.1	13.58
04-jul-93	58	5	81.44	0.77	49.51	9.4	15.31
06-jul-93	60	5	80.63	1.01	48.97	9	15.14
08-jul-93	62	5	81.04	0.74	64.89	7.6	20.06
13-jul-93	67	6	80.09	0.59	70.96	8	21.93
14-jul-93	68	6			49.34	8.5	15.25
17-jul-93	71	6			66.93	10.1	20.69

Date	day of growth	larval stage	respiration at 6°C (µl O2/h/ind)	mean protein content (µg/ind)	stddev protein content (µg/ind)
07-may-93	1	1			
09-may-93	2	1			
10-may-93	3	1			
12-may-93	5	1			
13-may-93	6	1	1.24	263.16	3.10
14-may-93	7	1			
16-may-93	9	2			
20-may-93	13	2			
21-may-93	14	2	1.37	377.89	6.10
22-may-93	15	2			
24-may-93	17	3			
26-may-93	19	3			
27-may-93	20	3	1.69	508.32	2.30
28-may-93	21	3			
30-may-93	23	3			
31-may-93	24	3	1.65	621.28	14.90
01-jun-93	25	3			
03-jun-93	27	3			
05-jun-93	29	3			
09-jun-93	33	4			
09-jun-93	33	4	1.81	725.16	21.00
11-jun-93	35	4			
13-jun-93	37	4			
15-jun-93	39	4			
16-jun-93	40	4	2.97	1196.76	30.00
17-jun-93	41	4			
18-jun-93	42	4			
21-jun-93	45	5			
21-jun-93	45	5	3.05	1337.51	3.80
23-jun-93	47	5			
23-jun-93	47	5	3.69	1595.12	7.50
25-jun-93	49	5			
28-jun-93	52	5			
28-jun-93	52	5	3.80	1919.35	43.80
30-jun-93	54	5			
30-jun-93	54	5	3.31	2184.92	68.90
02-jul-93	56	5			
04-jul-93	58	5			
06-jul-93	60	5			
08-jul-93	62	5			
13-jul-93	67	6			
14-jul-93	68	6	4.08	2738.20	56.30
17-jul-93	71	6			