Canadian Technical Report of
Fisheries and Aquatic Sciences 1272

April 1984

BROODSTOCK MANAGEMENT FOR YEAR-ROUND PRODUCTION OF LARVAE FOR CULTURE OF THE AMERICAN LOBSTER

by

S. L. Waddy and D. E. Aiken

Invertebrate Physiology Section
Fisheries Research Branch
Department of Fisheries and Oceans
Biological Station
St. Andrews, New Brunswick EOG 2XO
Canada

This is the one hundred and sixty-eighth Technical Report from the Biological Station, St. Andrews, N. B.

Cat. No. Fs 97-6/1272E ISSN 0706-6457

Correct citation for this publication:

Waddy, S. L., and D. E. Aiken. 1984. Broodstock management for year-round production of larvae for culture of the American lobster. Can. Tech. Rep. Fish. Aquat. Sci. 1272: iii + 14 p.

ABSTRACT

Waddy, S. L., and D. E. Aiken. 1984. Broodstock management for year-round production of larvae for culture of the American lobster. Can. Tech. Rep. Fish. Aquat. Sci. 1272: iii + 14 p.

A system for continuous production of larvae from mature (nonovigerous) females from the commercial fishery has been developed. Lobster larvae have been hatched and reared on a year-round basis in the St. Andrews Lobster Culture Facility for nearly a decade. For most of this period, wild-caught preovigerous females have been used, and egg production from such females has consistently been about 95%, with 85% of the resulting ovigerous females hatching according to an established schedule. Production of larvae from cultured stock has been accomplished for the first time, amounting to 21% of of our 1983 production.

Larval production from females spawning in the laboratory can be scheduled over a 12-mo period by holding the females on specific temperature regimes to regulate the rate of embryonic development and hatching time. Flexibility in scheduling is obtained through adjustment of temperature schedules and an appropriate mix of sizes of maternal females.

About 200 mature, cultured lobsters, grown rapidly in a high density culture system, were selected as broodstock for the development of domesticated strains. Lobsters grown at constant 20°C show poor reproductive performance; only about 5% of mature cultured females extrude eggs and cultured males have limited sperm and spermatophore production. However, environmental manipulation has increased fecundity in both sexes. Insemination problems such as impotence in males and unobserved molts by females are being circumvented with electroejaculation and artificial insemination techniques. Sperm can often be obtained from impotent but genetically valuable males for insemination of hard-shell females.

Our facilty now contains more than a thousand second generation lobsters derived from cultured parents selected from the thousands of animals hatched and reared to maturity under intensive culture conditions. These lobsters represent the first significant departure from the use of wild stock for lobster culture purposes, and are the first step toward development of a domesticated strain.

RÉSUMÉ

Waddy, S. L., and D. E. Aiken. 1984. Broodstock management for year-round production of larvae for culture of the American lobster. Can. Tech. Rep. Fish. Aquat. Sci. 1272: iii + 14 p.

On a mis au point un système pour la production continue de larves à partir de femelles matures (non ovigères) provenant de la pêche commerciale. Il y a maintenant presque dix ans que l'on fait éclore et que l'on élève des larves de homard à l'année longue aux Installations de culture du homard de St. Andrews. Pendant la plus grande partie de cette période, on a utilisé des femelles pré-ovigères capturées dans la nature; la production d'oeufs s'est maintenue constamment aux environs de 95 %, et 85 % de ces femelles devenues ovigères ont donné lieu à des éclosions selon un calendrier prédéterminé. Nous avons réussi pour la première fois à produire des larves à partir du stock cultivé. La quantité produite représente 21 % de notre production pour 1983.

La production de larves à partir de femelles qui se reproduisent en laboratoire peut être réglée sur une période de 12 mois en maintenant les femelles dans des conditions de température bien précise qui agissent sur la vitesse du développement embryonnaire et sur le moment de l'éclosion. On peut obtenir une certaine flexibilité en modifiant les régimes de température et en assurant un mélange approprié de femelles de tailles différentes.

Environ 200 homards matures qui ont été cultivés et qui ont connu une croissance rapide dans un système de culture de densité élevée ont été choisis comme stock de reproduction pour l'obtention de souches domestiquées. Les homards élevés à une température constante de 20 °C ont un piètre rendement du point de vue de la reproduction; environ 5 % seulement de femelles matures élevées ont pondu des oeufs et les mâles présentent une production limitée de sperme et de spermatophores. Toutefois, grâce aux manipulations du milieu, on est parvenu à accroître la fécondité chez les deux sexes. Les problèmes d'insémination telles l'impuissance chez le mâle et les mues ratées chez la femelle ont été résolus par des techniques d'électroéjaculation et d'insémination artificielle. Souvent on peut obtenir du sperme chez des mâles impuissants, mais génétiquement aptes, pour l'insémination des femelles à carapace rigide.

Nos installations comptent maintenant plus de mille homards de seconde génération provenant de parents élevés en culture choisis parmi les milliers de homards éclos et élevés jusqu'à maturité dans des conditions de culture intensive. Ces homards représentent la première tentative importante pour éviter l'utilisation des stocks sauvages à des fins de culture du homard et constituent le premier pas vers la production d'une souche domestiquée.

INTRODUCTION

In the last decade, many biological and engineering problems that originally confronted lobster culturists have been eliminated. However, progress in broodstock management has been slow. Although control of reproduction is an essential element of genetic studies and selective breeding for strain improvement and domestication, the reproductive biology of lobsters has been poorly understood. Only recently have we achieved an understanding of the reproductive cycle sufficient to determine maturation, control mating, predict egg extrusion and influence brooding success and hatching times (Aiken and Waddy 1980a,b,c,d, 1982; Aiken et al. 1984; Waddy and Aiken 1981, 1984). As a result, solutions are available for many of the broodstock problems that have been described recently (Hedgecock 1983; Hedgecock et al. 1978; Richards and Wickins 1979; Schuur et al. 1976; Talbot et al. 1984; Van Olst et al. 1980).

Lobster culturists have reported difficulty obtaining a high incidence of egg extrusion and successful egg attachment from females in captivity (Hedgecock 1983; Talbot et al. 1984), and have had to rely on wild caught ovigerous females for larval production. Since possession and sale of ovigerous lobsters are prohibited in the Canadian and U.S. fishery, the impossibility of this approach is obvious. Even if possession of ovigerous females were permitted, large-scale removal of ovigerous lobsters would generate strong resistance among fishermen, especially in areas where stocks are unstable or declining.

We have maintained a pilot-scale lobster culture facility for 10 yr and have developed methods for hatching larvae year-round to keep the facility in constant production. Larval production in the early stages of this project came exclusively from ovigerous females obtained from the fishery. With these females, techniques were developed for accelerating and retarding embryo development so that first-stage larvae could be obtained reliably in all months of the year. Eventually the supply of ovigerous females was supplemented with and gradually replaced by repeat-spawners maintained in the system. A method for identifying mature, preovigerous females was developed (Aiken and Waddy 1982) which made it possible to purchase barren but preovigerous females from the commercial catch in the spring, obtain new eggs that same summer with a minimum investment in care, terminating our dependence on the commercial fishery as a source of ovigerous females.

The ultimate objective of broodstock management is the development of a closed culture system utilizing only cultured stock. In this way, desirable culture traits such as rapid growth, efficient food conversion, social tolerance and disease resistance can be genetically enhanced. Progress is being made in this direction as well. We have 200 cultured broodstock males and females that have been selected from the thousands raised through the system and are rearing several thousand of their offspring. Techniques of electro-ejaculation and artificial insemination of intermolt females have been developed recently (Aiken et al. 1984) that help simplify the selective breeding process.

The following describes the basic elements of a proven system for continuous production of seed stock from mature (nonovigerous) females obtained from the commercial fishery. The approach is very different from that described by Hedgecock (1983) and is, in our opinion, simpler and more reliable. With this method we obtain a higher incidence of egg extrusion and larval hatching than was reported by Hedgecock and have solved one of the major problems he identified - difficulty obtaining year-round production of larvae.

BROODSTOCK MANAGEMENT STRATEGIES

USE OF WILD STOCK

For at least a few years, brood stock for new lobster culture projects will have to come from wild stocks simply because domesticated stocks are not available. There are two basic strategies involved in the use of wild stock: obtain ovigerous females from the fishery, or obtain preovigerous females and hold them until eggs are extruded. Each of these in turn yields a second pair of options: sell the female once her eggs have hatched or retain her for future egg production. Each of these approaches has legal and economic ramifications that will differ from one operation to another and therefore should be considered carefully before a particular approach is adopted.

Ovigerous Females

In most areas it is difficult to obtain ovigerous females legally from the commercial fishery. In reality, an ovigerous female removed from the grounds a week or a month after extrusion represents no greater loss to the fishery than a preovigerous female caught a week or a month before extrusion. But ovigerous females are protected by law, and permits to take them for culture purposes are difficult to obtain. In areas such as the Bay of Fundy, where lobsters are impounded in large tidal enclosures, females captured in the preovigerous state often extrude eggs in the impoundment. Although these females are a potential source for culture purposes we have found a high incidence of gaffkemia among such animals and we no longer purchase lobsters that have been held in any other facility.

Ovigerous females can be obtained from the fishery with either new ('black') or old ('brown') eggs, depending on locale and season of year. Since females from different areas of the fishery spawn at different times, the various fisheries offer considerable latitude for selection. Old eggs can only be used for larval production in the spring and early summer. Development of new eggs obtained during summer and fall can be accelerated or retarded with temperature according to the relationships described by Perkins (1972) so that hatching will occur throughout the winter and the following spring, summer and autumn. As might be expected, the months from October to January are the most difficult in which to sustain hatching of larvae. Failures occur from a variety of causes (female death, egg loss, disease, predation by nemerteans, premature molt of the female) which seem to be exacerbated by the prolonged abnormal temperature exposure required to induce hatching during this period.

Preovigerous Females

Preovigerous mature females can be purchased from the commercial fishery with no legal complications, and held in a culture system until eggs are extruded. This approach has been attempted in California with females captured in the autumn after mating, with extrusion rates of 60-80% (Hedgecock 1983, Hedgecock et al. 1978, Schuur et al. 1976, Van Olst et al. 1980), but such females usually will not extrude eggs until the following summer, and must therefore be maintained for many unproductive months.

An alternative is to purchase females in the spring, before the spawning season, on the assumption that those not carrying eggs are preovigerous and will spawn within a month or two. Females offered for sale often include a significant proportion of immature ones and others from which the eggs have been lost for a variety of reasons. Such females will not spawn for another year and unless they can be identified and eliminated, this approach makes little economic sense.

Fortunately, the endopodite cement glands are an excellent indicator of ovary condition and we have recently described a technique for prediction of egg extrusion, using these glands (Aiken and Waddy 1982). With this method, preovigerous females can be easily selected from the commercial catch in the spring and need only be held for 1-2 mo before eggs are extruded. Advanced cement gland development (stages 3 and 4), indicative of impending oviposition, is obvious on gross examination with the unaided eye.

We routinely obtain preovigerous females in late May or early June from the southern Gulf of St. Lawrence, for egg extrusion in July. By simply maintaining these females at local seawater temperature and photoperiod, extrusion will occur between late June and mid-August, with 80% of females spawning during the first 3 wk of July. These lobsters are 80-90 mm CL and carry an average of 8000 eggs. Using this method, we obtain an extrusion rate of 96%. It should be noted that females very close to spawning will resorb their ovary if exposed to the stress of handling and shipping. We have had no problem with females captured and transported 6-8 wk ahead of predicted extrusion time, whereas up to 30% of those obtained within 2 wk of spawning have reacted with massive ovarian resorption. So the only constraint appears to be the requirement that preovigerous females be captured and shipped at least a month before oviposition.

Assuming 4% of the preovigerous females selected from the fishery fail to spawn successfully, and 16% of the resulting ovigerous stock will not hatch on schedule (Fig. 1), a culture facility using this system should obtain approximately 20% more than the calculated number of brood females to sustain the system, and schedule the extras for the difficult autumn and early winter period.

To determine how reliably year-round larval production could be obtained in a culture facility we implemented a rigid production schedule over a 6-yr period. Newly spawned females were assigned to a schedule designed to yield 1000 fourth stage lobsters every month. For this evaluation, individual temperature schedules were not altered once set, and no attempt was made to compensate for death, disease or mechanical failure by bringing in additional females. Success in meeting larval production schedules between 1976 and 1982 is shown in Fig. 2.

In the first 3 yr of operation, we had difficulty meeting production levels from October through January. By defining the problems that regularly occurred during the autumn and winter, we were able to compensate for them by scheduling additional females for this time and, since 1979, have virtually eliminated that period of low larval production.

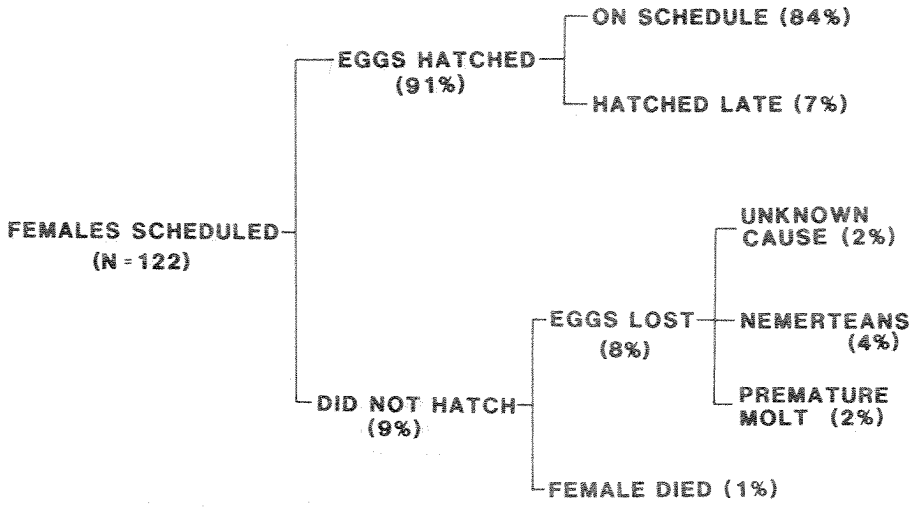

Fig. 1. Summary of results from 122 ovigerous females scheduled to hatch larvae over a 36 mo period in a culture facility.

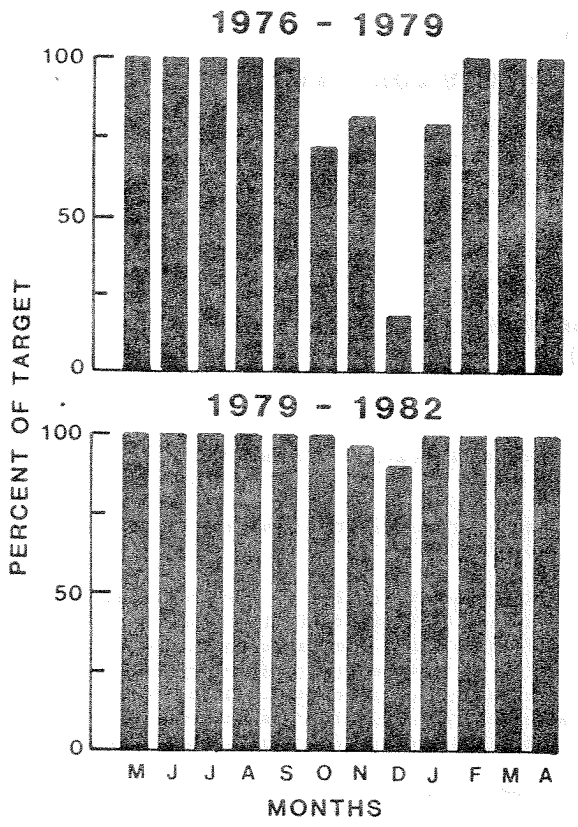

Fig. 2. Success in meeting larval production schedules in the Lobster Culture Facility from 1976-1982, using the methods described in this paper. Target level of 100% is 1000 fourth stage larvae produced each month.

Repeat Spawners

Because of the extended reproductive cycle (2 yr) and cost of holding space, it is seldom advisable to retain a wild female after her eggs have hatched. However, the occasional female (or her offspring) will display desirable culture characteristics and it may be worth retaining the female for future larval production.

The most effective way to accomplish this is to return the female to the equivalent of an East Coast water temperature and photoperiod cycle. Molting will occur after the eggs have hatched and the female can then be inseminated naturally or artificially (Aiken et al. 1984).

Most female lobsters spawn between July and September of alternate years with hatching, molting and mating occurring during the intervening summer. More than 98% of wild females in our facility adhere to an alternate year molt and egg extrusion pattern. Under our local environmental conditions, about 90% of egg extrusions from wild females occur during July and, if these ovigerous females are maintained under local seawater temperature, hatching occurs the following summer.

It is significant that reproductive cycles reported for American lobsters maintained under conditions north of San Francisco, California, differ from those described here (Hedgecock 1983; Nelson et al. 1983). They describe an irregular cycle in which molting occurs more frequently than spawning and one in which there is a pronounced lack of seasonality in reproductive events with egg extrusion occurring from February to December. As well, many of the females do not extrude eggs within every molt cycle, producing 'anovulatory molts'. This is a reproductive cycle that we have not observed under our local temperature conditions but is one that becomes common when wild females are exposed to abnormal temperature regimes, particularly one with winter temperatures above 8-10°C (Waddy and Aiken 1984). These differences emphasize the effect of varied environmental conditions on lobster reproductive cycles.

Both Hedgecock (1983) and Talbot et al. (1984) have found the production of eggs and larvae to be extremely low from laboratory-spawned females, egg loss amounting to 70% of spawned eggs. This contrasts with the 90% successful hatching we have obtained from 403 controlled matings in our facility (Fig. 3). The reasons for the differences are not easily determined. One possibility is temperature: minimum winter temperatures in their laboratories are at least 10°C higher than the 0°C reached in our facility in late winter. In controlled temperature studies on females with established reproductive cycles, we found the incidence of egg extrusion to be reduced by about 75% when water temperature was not allowed to drop below 15°C in winter, by almost 50% when minimum temperature was 12°C and a 10% reduction when the minimum temperature was 7°C (Waddy and Aiken 1984). We have also found that females maintained at other than normal temperatures during ovarian development suffer higher rates of egg loss than those held under local seawater temperatures.

For these reasons, and the fact that little is known about the interrelationships among temperature, photoperiod, ovary development, oviposition, and egg attachment, we recommend that brood stock be returned, after hatching, to a photoperiod and temperature regime close to that occurring in the lobster's natural range, particularly that winter temperature be reduced to less than 5°C. In our area, the period of low temperature lasts for 4.5-5 mo from mid-December until late April or early May (Fig. 4). It would be beneficial if the length of the cold period could be reduced somewhat with a slightly accelerated year. Our preliminary experiments indicated that 3 mo of low temperature were adequate (Aiken and Waddy 1976); but the full effects of a compressed year have not been determined and at present we do not propose its use for routine production.

At this point we would not recommend repeat spawners as a method for supplying all the larvae required by a facility because the alternate-year reproductive cycle requires that more than twice as many females be maintained as would otherwise be required, and an additional stock of males must also be maintained for mating. At present, the most economically viable method is to purchase healthy preovigerous females directly from the fishery each spring, obtain both extrusion and hatching in the facility, and then sell the females.

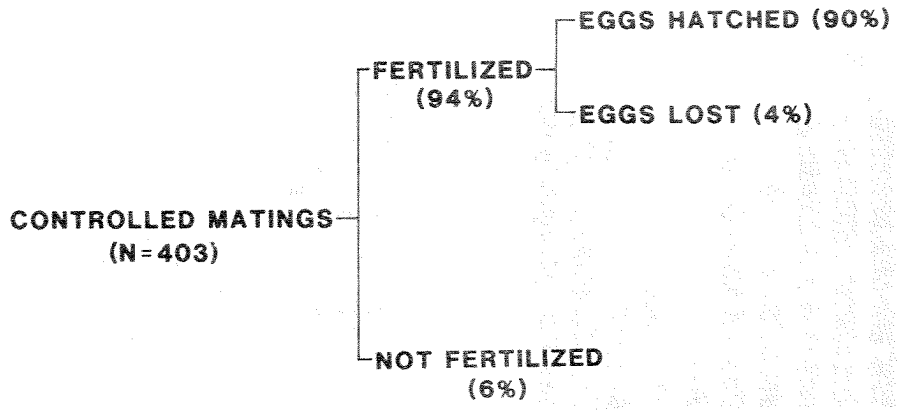

Fig. 3. Results of 403 controlled matings with broodstock females retained for a a second cycle of larval production over a 7-yr period.

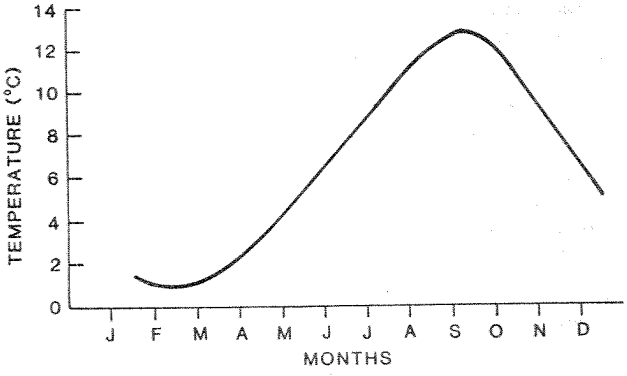

Fig. 4. Annual cycle of seawater temperature at St. Andrews, N.B. Curve is mean monthly average over a 4-yr period.

USE OF CULTURED STOCK

Lobsters hatched and reared in a culture system are subject to severe selection pressure. Those that survive and grow rapidly to maturity can be considered better adapted to culture conditions and it seems reasonable to expect that some of this tendency toward survival and growth under culture conditions is heritable. It is therefore important that methods be developed for obtaining progeny from lobsters that have reached maturity in a culture system. We have been addressing the problems of obtaining eggs and sperm from cultured lobsters for the past 5 yr and now have more than 200 mature cultured broodstock animals in our facility, selected from the thousands reared through the system. These cultured lobsters have tolerated confinement, resisted disease and have grown rapidly in a high density holding system. Future progress in strain development will depend on the extent reproduction in cultured lobsters can be controlled.

Reproductive cycles of cultured females reared at constant 20°C are typical of those described for wild females under similar temperature conditions. Spawning is rare, molts occur every 4-12 mo and oocyte resorption is common. Only about 5% of females extrude eggs in constant 20°C and uncontrolled photoperiod (Waddy and Aiken 1984).

One of the problems caused by constant high temperature is increased molting frequency. Constant 20°C temperature decreases the intermolt time in mature females from 2 yr to as little as 4 mo. Because of shortened intermolt times, the precise phasing of molt and reproductive cycles has little chance of occurring. Indications are that photoperiod in autumn may be the synchronizer of final vitellogenesis and egg extrusion. But whatever the inductive event, it is effective only at certain molt stages. Premolt events and final vitellogenesis are incompatible (Aiken and Waddy 1976) so the induction of vitellogenesis must occur prior to D_0. Under culture conditions (20°C) the occasional female does extrude eggs, suggesting the coincidence of an inductive environmental event with the appropriate molt and reproductive stage. Timing of molt and reproduction is rarely a problem with female lobsters held in East Coast seawater temperatures. Low winter temperature appears to synchronize the molt and reproductive cycles so ovarian maturation can proceed without interference.

Our present strategy for maximizing larval production from cultured stock is to transfer mature-sized males and females to a temperature and photoperiod regime close to that experienced by wild populations. Conditioning both males and females in a 'winter' environmental regime where the temperature drops below 5°C for 4-5 mo increases the fecundity of both sexes, and preliminary data indicate that egg production by cultured females may approach that of wild stock after three seasonal cycles (Fig. 5).

Egg attachment and incubation success are not as great in cultured females as in laboratory-held wild females. Only 43% of cultured females retained more than an estimated 75% of their eggs to hatch

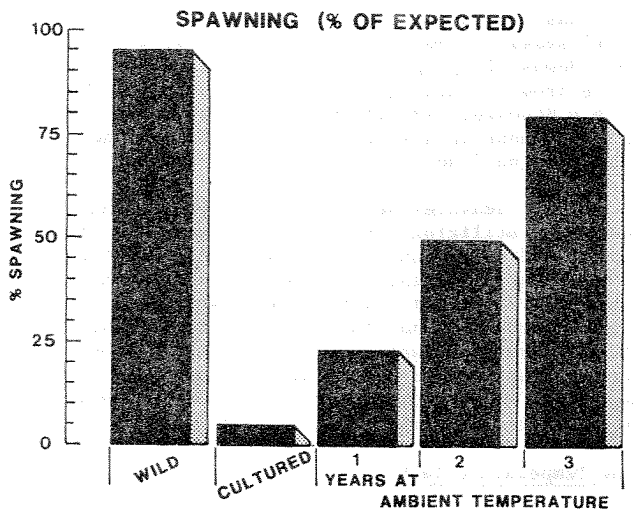

Fig. 5. Effect of transferring mature cultured females to a local temperature and photoperiod regime. After 3 yr under ambient conditions egg extrusion approaches that obtained with wild stock.

(Waddy and Aiken, in prep.). A similar degree of egg loss has been obtained with wild females exposed to high winter temperatures.

Despite the problems in producing seed stock from cultured lobsters, progress has been impressive. As recently as 1981, fertilized eggs had not been obtained from a cultured female. During 1983, 21% of our fourth stage larval requirements were produced by cultured parental stock. These larvae are the first reported from culture-culture crosses and represent the beginning of a domesticated strain of American lobsters.

Recognizing Maturity

Females must be mated if their first egg mass is to be fertile, and most successful matings occur immediately following molt. Since lobsters of a mature size are usually held individually, it is important that onset of maturity be recognized so that females can be mated.

Body size is not a reliable indicator of maturity since females can mature over a wide range of sizes. However, the 'Maturity Index', or ratio of abdominal width to carapace length is reasonably useful (Aiken and Waddy 1980a). With this simple determination, maturing females in the culture system can be identified and moved to conditions suitable for mating and ovary development.

Even more useful as an indicator of maturity are the cement glands on the pleopod endopodite. These cement glands wax and wane with ovarian development, and the presence of well developed glands on a nonovigerous female is conclusive evidence of maturity and impending oviposition. Well developed cement glands can be easily detected on the preovigerous female and at this time she can be inseminated artificially if her previous molt was missed.

Male maturity is more difficult to assess, although the crusher propus index (CPI) has proven useful for wild males (Aiken and Waddy 1980d). Because of the reduced crusher claw development in many individually-reared males, the method is limited as a maturity index for cultured males. At present there is no reliable way to determine maturity of most cultured males except by spermatophore production and mating capability. This presents a major problem to the breeding program as there may be considerable variation in size at first maturity.

Controlled Mating

Intermolt mating occasionally occurs naturally (Aiken and Waddy 1980a,b; Dunham and Skinner-Jacobs 1978), but the stimulus for it is not known, and results have been unpredictable. To ensure natural mating the male and female must be together during or shortly after the female molt. Some wild male lobsters held for more than a year under laboratory conditions become impotent (Aiken et al. 1984) and prove useless for mating. We have found that if mating does not take place within 15 min, it is expedient to substitute another male. If copulation is not observed directly, the presence or absence of a spermatophore in the seminal receptacle can be determined. Immediately after mating, a portion of the spermatophore is often visible externally. If this is not the case, the spermatophore can be seen by gently opening the receptacle with 'barraquer' forceps or, once the spermatophore is hardened, it can be detected by gently probing the receptacle.

Molted lobsters may not be detected in individual containers in a large culture facility. If the female's brief receptive period has passed, up to 2 yr will elapse before the opportunity for mating is again presented. To alleviate this problem we recently perfected electroejaculation and artificial insemination techniques (Aiken et al. 1984). Sperm can often be obtained from impotent but genetically valuable males and used to inseminate intermolt females.

So far, we have had two instances of successful fertilization, attachment and hatching of eggs from artificial inseminations. The cultured females, inseminated at molt stage C_4, establish the feasibility of the technique. We now have 120 cultured females that are artificially inseminated and are due to spawn next summer. These females ranged in molt stage from A to C_4 at insemination and the results should give an indication of the potential of artificial insemination for fertilizing postmolt and intermolt females.

Influence of Temperature

The culture temperature in most facilities is maintained at 20-22°C to promote rapid growth. At these temperatures some females will reach maturity when less than 60 mm carapace length and when approximately 18 mo of age.

Unfortunately, more than 95% of females held at constant 20°C resorb their oocytes instead of extruding eggs (Waddy and Aiken 1984). Other work indicates that a period of 'winter temperature' conditioning is essential for ovary development and normal incidence of spawning, both in wild and cultured stock. Elevated winter temperature above 8-10°C disrupts the normal cyclic relationship between molting and egg extrusion and generally leads to increased rates of molting and a reduced incidence of spawning (Waddy and Aiken 1984).

Because of these results, it is essential to return potential broodstock to a normal environmental cycle after the pubertal molt.

As well as the problem with egg extrusion, male fecundity is also adversely affected by constant high temperatures. Cultured males at constant 20°C produce few spermatophores, and those that are produced are often too small to be implanted or else lack a sperm tube. Once these cultured males are transferred to conditions of ambient temperature and photoperiod, spermatophore and sperm production increase to more acceptable levels (Aiken et al. 1984). Holding conditions also appear to influence spermatophore production as males recently brought from the wild routinely produce more than twice the number of spermatophores than males that have been laboratory-held for more than a year in individual holding (Aiken et al. 1984).

Influence of Photoperiod

Although Hedgecock (1983) and Nelson et al. (1983) maintain that photoperiod is an effective means of regulating the time of oviposition, our results indicate the situation is not that straightforward. While they found that an artificial extension of winter lighting conditions allows the scheduling of spawning for late fall and winter, our results are diametric to this. We conducted two experiments involving direction of change, absolute length and time of onset of spring photophase and found no effect of spring photoperiod on the onset of final vitellogenesis or timing of egg extrusion (Aiken and Waddy, in prep.). One critical difference between the experiments here and in California is water temperature. Our experiments in which mature females were exposed to elevated winter temperatures (Waddy and Aiken 1984) produced reproductive cycles similar to those obtained at Bodega Bay and indicate their results are confounded by high local temperatures and that firm conclusions on photoperiod effects should not be drawn without further replication. But it is clear that, under our local environmental conditions, time of spawning cannot be manipulated with spring photoperiod. However, we do not rule out a photoperiodic influence and feel that it might be a factor prior to the winter solstice of the year preceding egg extrusion. Experiments are under way that may help define the role of autumn photoperiod. In addition, recent preliminary work idicates that spring photoperiod, while not affecting egg extrusion, does have an effect on the timing of the molt that occurs in the year prior to spawning. Females exposed to to short days throughout the winter, spring, and summer molted much later in the year than normal and some did not molt at all. Although these results are not definitive, they indicate that more work needs to be done on the effects of photoperiod on reproductive and molting cycles.

TECHNIQUES AND PROBLEMS

HOLDING FACILITIES

Temperature Control

Three temperatures were used in the broodstock system: local seawater (0-14°C), standard culture temperature (20°C), 'cold' (2-4°C). Cold temperature was obtained with individual refrigeration units as described below. Culture temperature of 20°C was obtained by mixing 'ambient' (0-14°C) and 'hot' seawater (30°C) with a pneumatically operated Dahl 3-way PVC proportional mixing valve. Temperature from the mixing valve was maintained at 20°C with a Honeywell RP908A pneumatic controller and LP914A sensor in a stainless steel well in the main distribution line.

'Hot' seawater was obtained by preheating local seawater, utilizing a two-step process. The first step involved passing the incoming seawater through a titanium plate heat exchanger against fresh water heated in 340 m^2 of solar panels (two-phase thermosyphon type manufactured by Nortec Solar Ltd). The pre-heated seawater was then routed to the second step, an oil-fired multiple-tube heat exchanger that ensured a constant 30°C for seawater directed to the mixing valve (Fig. 6).

Low Temperature Tanks

Embryonic development was protracted by holding ovigerous females at 2-4°C. Females were held in individual compartments in trays stacked vertically in an insulated FRP well approximately 75 x 75 x 135 cm deep (Fig. 7).

Water was recirculated within the well by an airlift pump and refrigerated with a model D1-100 Min-O-Cool (Frigid Units Inc.) modified to pump refrigerant through 16 mm flexible UPC hose 3 m to a pair of 15 m titanium coils in the bottom of the well. New seawater at local temperature was added continuously at 0.3 L/min.

Standard Brood Tanks

Females were held at local seawater temperature or at 20°C in single or multiple compartment brood tanks in which water flow of 2 L/min was directed along the floor. Tanks were construced of 2 cm plywood with three coats of epoxy paint. The front face of the tank was 12 mm clear acrylic plastic to facilitate observation (Fig. 8).

Hatching Tanks

At late stages of embryonic development ovigerous females were transferred from brood tanks to hatching tanks. Effluent in these tanks was directed through a screened basket that removed and concentrated hatched Stage I larvae (Fig. 9). Larvae collected in this way were transferred to standard 40-L Hughes planktonkreisels (Hughes et al. 1974).

CONTROL OF HATCHING TIME

Egg development rate varies according to temperature, salinity, age of the eggs, temperature history, health of the eggs, and other conditions. We have found the Perkins Index (Perkins 1972) can be used to predict hatching time within 4 d as much as 6 wk in advance when healthy eggs are held at constant temperatures. Eggs that are held for extended periods at less than 5°C will hatch 7-10 d earlier than predicted from the Perkins Index. Eggs that become infected with the microbial epibiont, Leucothrix, will sometimes hatch considerably later than predicted from the Perkins Index. The procedure for regulating embryonic development rate and hatching time with temperature is as follows (Fig. 10):

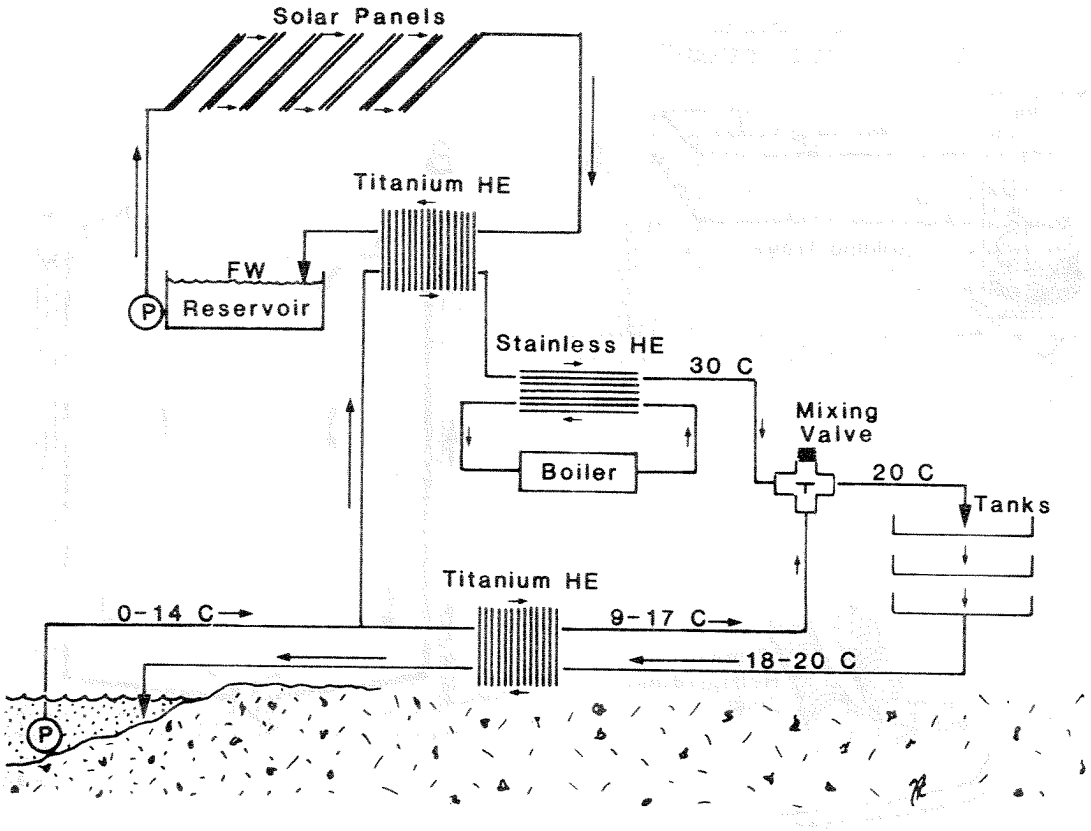

Fig. 6. Schematic of system used to raise seawater from local 0-14°C to 20°C for lobster culture at the Biological Station, St. Andrews, N.B.

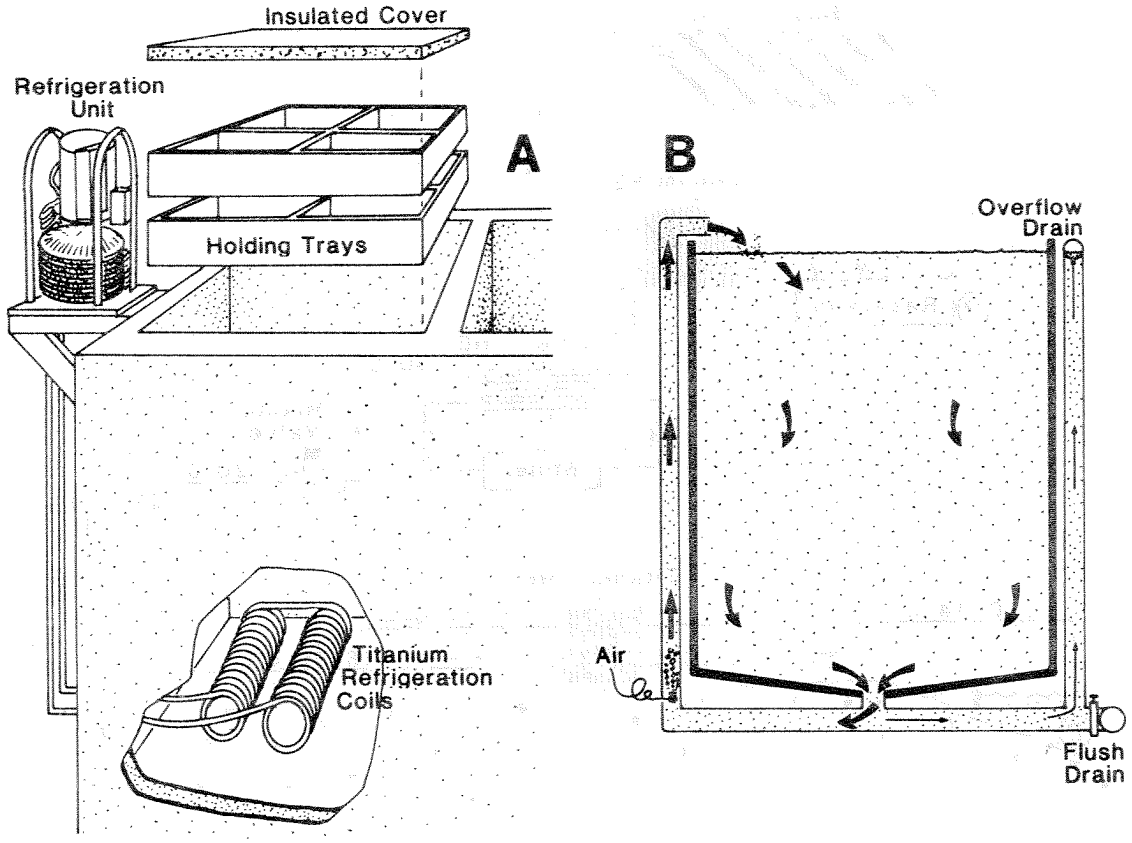

Fig. 7. Low temperature recirculation tanks for delaying embryonic development on ovigerous female lobsters. A. View of holding trays, insulated tanks and refrigeration system for maintaining water at 2-4°C. B. Schematic of water circulation in the tanks. Arrows indicate direction and relative proportion of flow. A small quantity of new water is added continuously.

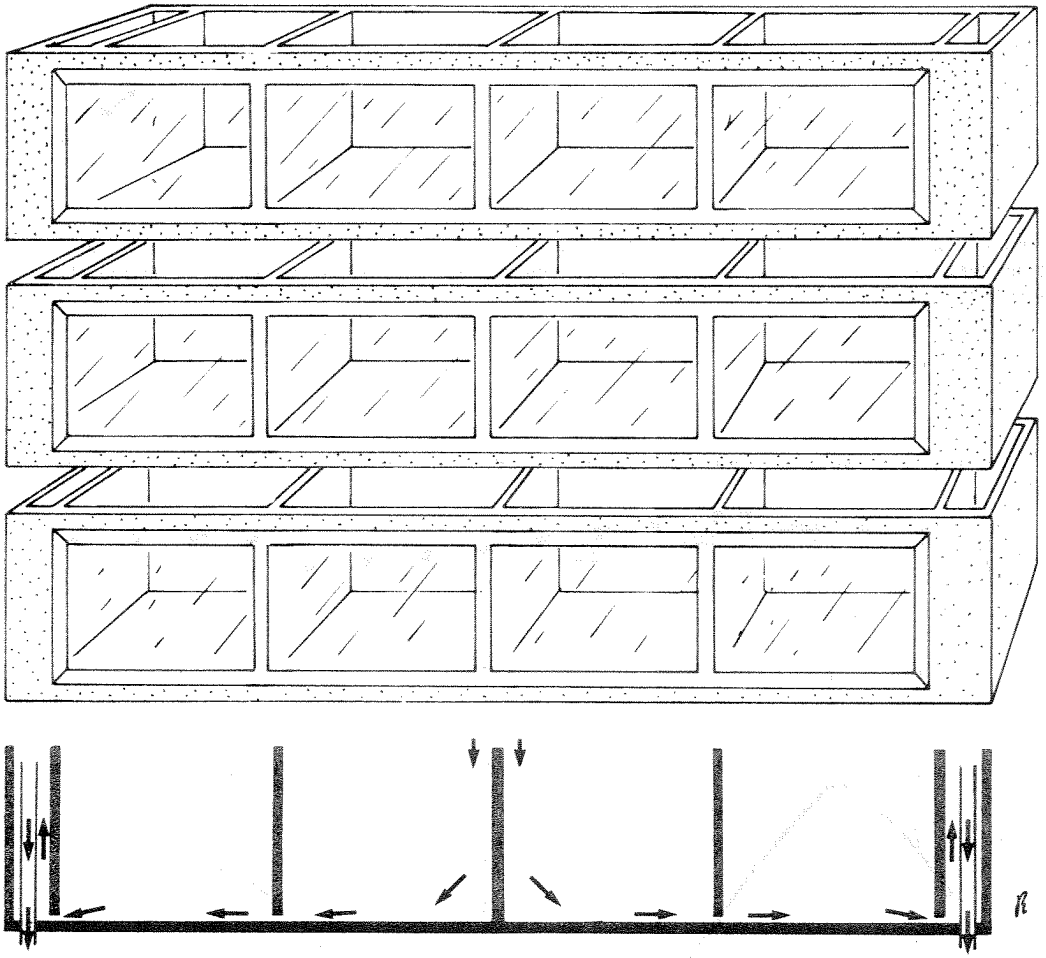

Fig. 8. Standard brood tanks for holding ovigerous females. Various sizes and compartment configurations exist but all incorporate the basic features of clear acrylic front panel, laminar flow of water along tank bottom (illustrated in bottom section) and flushing-cleaning action activated by removable standpipe.

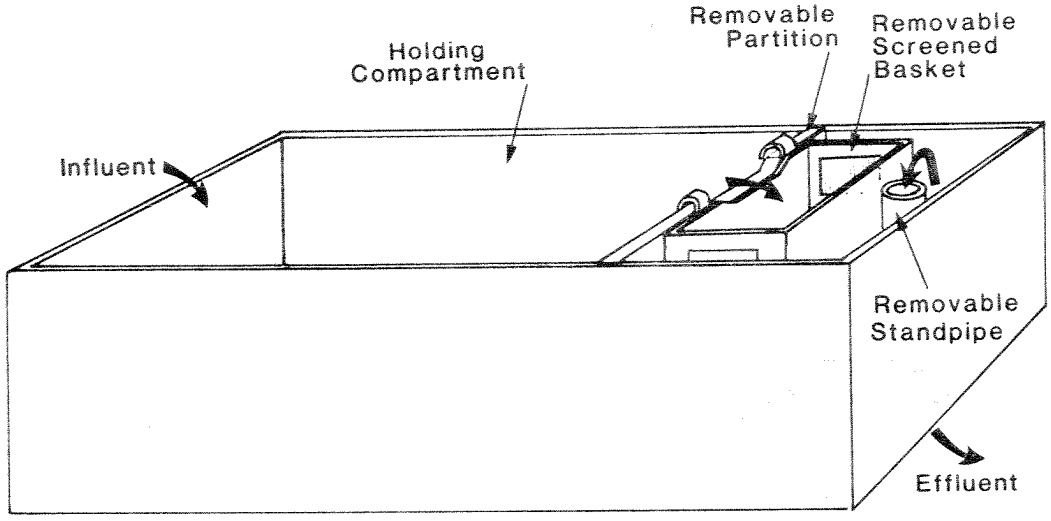

Fig. 9. Hatching tank for collecting first stage larvae. Water flow carries hatched larvae from the holding compartments to the screened basket where they are retained until transferred to planktonkreisels.

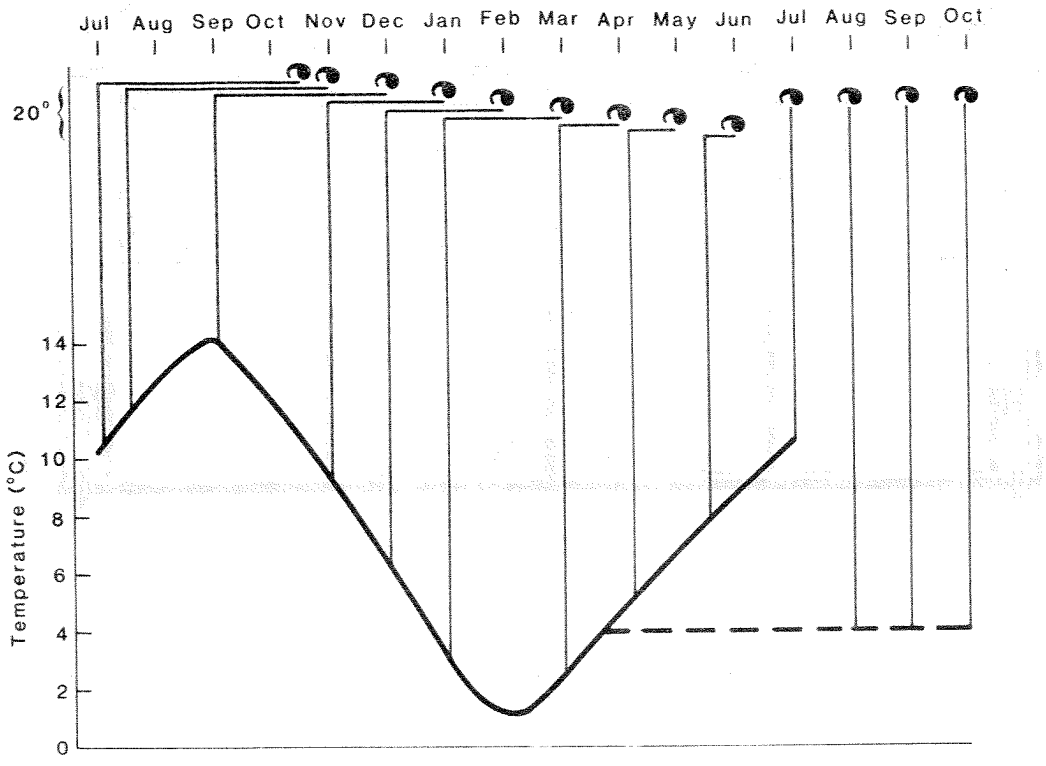

Fig. 10. Procedure for year-round production of lobster larvae. Females with newly spawned eggs are held at local seawater temperature (heavy line) or in water cooled to < 5°C (dashed line) and transferred to 20°C according to estimates made with the Perkins Index. Larval symbols indicate hatching in the appropriate months.

(a) To obtain larvae in mid-to-late October - females that spawn in late June or early July are held at 20°C until the eggs hatch.

(b) To obtain larvae from November through July - ovigerous females are held at local seawater temperature until the appropriate time as estimated from the Perkins Index and then held at 20°C until hatching.

(c) To obtain larvae from August through October of the following year - ovigerous females are held at local seawater temperature through autumn and winter, then at 2-4°C until the Perkins Index indicates they should be transferred to 20°C to obtain hatching at the appropriate time.

With our local seawater temperature this method requires only facilities to cool water to 2-4°C, in addition to local seawater and normal system culture temperature (20-22°C). Temperature below 5°C can be obtained with an ordinary seawater refrigeration system and requires no expensive temperature mixing capability. In localities where local seawater temperature is different from that shown in Fig. 4, some adjustment to the development schedule will be necessary.

Larvae can be obtained from September through December only by maximum acceleration or maximum retardation of embryonic development. Both are extreme conditions that contribute to reduced success in producing larvae during these months. Development is most easily retarded in new eggs, as those close to hatching will develop slowly even at temperatures lower than 5°C. Although retardation is most successful in early stages, it can be used at any time for prolonging development time. We recommend that a strategy be chosen and then be strictly adhered to for each female. Increased manipulations and temperature changes increase the risk of female death or egg loss. Both methods (acceleration and retardation) to produce larvae during the autumn should be employed to improve the chances of hatching success during this difficult period. In addition, 15-20% more than the requirement of females should be scheduled for this time period.

ESTIMATING FECUNDITY AND DETERMINING SIZE OF BROODSTOCK REQUIRED

Continuous culture requires that larvae be produced at regular intervals, and in specific quantities. Production that exceeds the carrying capacity of the larval system is wasted. Insufficient production results in poor utilization of the facilities and an uneven flow of larvae through the system.

Time of hatch, as discussed above, can be controlled by temperature, but number of eggs is a function of female body size. Because of the logarithmic relationship between size and fecundity, one female lobster of 140 mm carapace length can produce five times as many eggs as one female of 80 mm carapace length. Since one large female can be housed in a smaller area than five small females, and requires less food, it might appear sensible to rely on a few large females for larval production. Unfortunately, the loss is also proportionately greater if a large female dies or loses her eggs (Table 1).

A variety of values exist for lobster fecundity (Aiken and Waddy 1980a). We have found that Perkins' (1971) fecundity values generally are within 10-20% of the actual number of first stage larvae in our system, and is sufficient to permit the type of manipulation described here.

An estimate of the required size of broodstock for commercial culture can be made using the figures obtained in our laboratory over the past 10 yr. Assuming 50% mortality during both larval and juvenile stages, 4 million first stage larvae must be hatched each year to produce 1 million market lobsters on a continuing basis (Fig. 11). Depending upon the size of broodstock females chosen (Table 1), the number of females needed to produce 4 million larvae can be estimated.

Also of major consideration is the size of the broodstock facility. Because of the October to October hatching schedule for each year's egg production, there is an overlap of 5-6 mo (May-October) when broodstock for 2 yr must be maintained. This means a 42-50% increase in the number of cubicles required for broodstock. For broodstock of 80 mm carapace length, 989 cubicles are needed, whereas for 140 mm females only 173 cubicles are required (Table 1).

It is most prudent to maintain an assortment of sizes. If a disaster kills several small females scheduled to produce in subsequent months, it is possible to fill the breach by accelerating a large female. If the normal capacity of the larval system is temporarily reduced, larval output can be adjusted by accelerating small females and retarding larger ones. The key to this is a proper blend of temperature manipulation and fecundity.

LOSS OF EGGS

Extruded eggs are attached by some ill-defined process to the non-plumose setae of the pleopods and ventral abdominal sterna. Attachment is usually secure, but occasionally an entire egg mass will be lost before hatching. In the wild, natural attrition of eggs carried by offshore females has been estimated at 36% (Perkins 1971). There are many causes for excessive egg loss, and disease, parasitism and unfavorable social conditions are most commonly cited. A few causes are relatively common and can seriously upset a production schedule unless recognized and controlled. Fortunately egg loss is a minor problem in our system. Wild females that successfully extrude eggs will almost always hatch their full brood. There is a greater incidence of egg loss among cultured females but the data on this are still very meager.

High incidence of poor adhesion and egg loss among females held at culture facilities in California (Hedgecock 1983; Talbot et al. 1984) suggests there may be environmental factors involved in egg retention. The fact that our cultured lobsters retain fewer eggs than wild ones also supports this. Preovigerous females in our facility are maintained under simulated local photoperiod and local seawater temperature, and egg attachment is highly successful.

Unfertilized Eggs

Unfertilized eggs either will not attach at all or will attach and be lost in succeeding weeks. This can be a problem when preovigerous females are

Table 1. Size of broodstock required to produce 4 million first-stage larvae per year.

CL (mm)	Thousands of first-stage larvae	Number of females hatching	Number of females scheduled	Number of preovigerous females	Number of broodstock cubicles	Percentage of years hatch per female
80	7	571	672	708	989	0.18
90	10	400	471	496	692	0.25
100	14	286	336	354	494	0.35
110	18	222	261	275	384	0.45
120	24	167	196	207	289	0.60
130	30	133	156	165	230	0.75
140	40	100	118	124	173	1.00

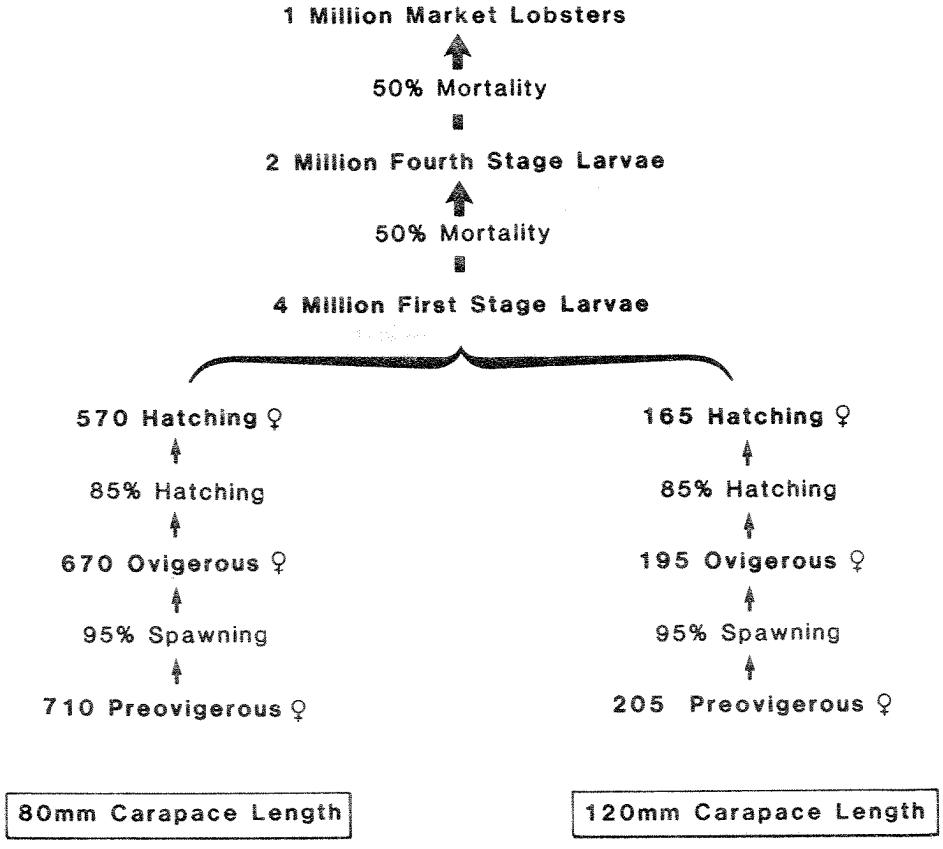

Fig. 11. Number of preovigerous broodstock females to be obtained annually from the fishery to supply a commercial culture facility producing a million market lobsters (0.45 kg) per year. Data are given for two sizes of broodstock females.

obtained from stocks with a significant proportion of unmated females (see Krouse 1973). This condition can be identified in preovigerous females by gently probing the seminal receptacle: an inseminated female has a sperm plug that blocks the entrance to the receptacle, and an uninseminated female does not. Extruded eggs that are insecurely attached can be examined microscopically for the cleavage planes indicative of embryonic development.

Hedgecock (1983) suggests that egg loss may be due to low or seasonal fertility of male lobsters. We have not found this to be a problem with wild males, but cultured males, particularly those at 20°C, often produce spermatophores devoid of sperm. These have been observed at electroejaculation but it is not known whether such a male would mate naturally or not. This is not a problem with wild males and has not been a factor with natural matings of wild stock (Fig. 4).

Micropredators

In 1978 we found large numbers of nemerteans (Pseudocarcinonemertes homari) on the egg mass of a wild ovigerous female in our culture facility (Aiken et al. 1983). This nemertean can destroy the eggs of both the American and European lobsters (H. gammarus) and infestation can occur at any time during the year-long incubation period. P. homari is found on lobsters thoughout Canadian Maritime waters with the highest prevalence in the Bay of Fundy around Grand Manan (J. Brattey, unpublished).

Infestation on ovigerous females can proceed in two ways: either the egg mass is totally destroyed by nemerteans consuming lobster egg yolk or the female lobster responds by gradually removing her entire egg mass. In either case, the result is total destruction of the lobster eggs. The only time the lobster's eggs survive long enough for larvae to hatch is when the infestation occurs sufficiently late in the incubation period so there is not time for all the eggs to be consumed. We have been fortunate in keeping the nemertean confined to wild stock but our recent discovery that the nemerteans can kill lobsters by totally destroying the gills if they are denied access to lobster eggs makes the problem for lobster culturists even greater.

Diseases

A number of diseases are known to affect lobster eggs (for reviews see Fisher et al. 1978; Rosemark and Conklin 1983; Sindermann 1977). Microbial epibionts such as Leucothrix are particularly troublesome because development of the infection is enhanced at temperatures (20-22°C) used to accelerate embryo development, and can transfer to the larva at hatching. Leucothrix infections can prolong embryo development by as much as 6 wk at 20°C, and can cause total egg mortality. Leucothrix can be controlled with neomycin (Fisher et al. 1978), and we have had good success with a 1-3 min dip of the female abdomen and attached eggs in a 5-10 ppt solution of the commercial iodophore, Wesdodyne. This treatment, once or twice a week at 20°C and less frequently at lower temperatures, restores a normal rate of embryo development and permits hatch with apparently normal larval viability. Harper and Talbot (1984) have described other bacteria on lobster eggs but could find no correlation between degree of infection and egg loss.

Shell disease can be a problem, particularly with valuable cultured brood stock. We have found that Wescodyne applied full strength to the affected area appears to limit the spread of the disease.

DISCUSSION

The larval production methods described in the preceding sections were developed during a decade of operation of a small integrated lobster culture facility in which year-round monthly production of larval lobsters was of paramount importance. Hedgecock (1983), in a recent review of seedstock production methods for the American lobster, said "Presently, techniques for truly continuous production of larvae are not available ...". We disagree. In this report we have described a technique we have been refining for 6 yr and with which we now obtain continuous larval production.

In the method proposed by Hedgecock (1983), photoperiod, not temperature, is used to induce vitellogenesis. This requires that females be held for a considerable time before egg extrusion, and that expensive holding space therefore be occupied throughout the preovigerous period. At the time the paper was written Hedgecock had carried only 39 females through all three steps of the proposed method, and only 60% of these had spawned. In the method we describe here 519 preovigerous females have been processed and 96% have spawned successfully with minimal requirement for holding facilities and maintenance prior to spawning.

Hedgecock and colleagues (see Nelson et al. 1983) have dismissed temperature as a significant factor in the induction and synchronization of vitellogenesis and spawning ('... temperature is not the factor controlling oviposition' - Hedgecock 1983). In a commentary on broodstock problems in lobster culture Hedgecock (1983) noted that only about half of the females that successfully lay eggs in their laboratory carry those eggs through to hatching, and in many of those the clutch size is reduced to only a few hundred eggs by the end of embryonic development. Wild and retained broodstock that spawn in our system consistently carry a full complement of eggs through to hatching. We feel the difference is in the temperature regimes used at our respective laboratories. We use 4-5 mo of winter temperature less than 5°C to synchronize vitellogenesis and spawning, whereas they use a much higher winter temperature and rely on photoperiod control to synchronize vitellogenesis and spawning.

In Hedgecock's (1983) opinion, '... reproductive events can be controlled and scheduled with remarkable accuracy using photoperiod'. Experiments we have conducted suggest that precise control can only be achieved when both photoperiod and temperature are combined, and that as winter temperature increases above approximately 6-7°C the precision declines and the incidence of reproductive failure increases. We suspect the problems that Hedgecock and and colleagues have encountered using wild lobsters as broodstock result from their use of winter temperature above this minimum.

Routine production of seed stock for culture of the American lobster is now possible with both wild and cultured stocks. Preovigerous wild females can be selected, on the basis of cement gland stage, from the commercial fishery in the spring and will

produce eggs in 1-2 mo under ambient conditions. These ovigerous females can be placed on a temperature regime that will regulate embryonic development and ensure on-schedule production of larvae all months of the year. In the 10 yr we have been using this system, egg extrusion, fertilization and successful attachment have occurred in 95% of females. Of those assigned to a year-round production schedule, 85% have produced larvae at the scheduled time.

Methods for the continuous production of larvae from broodstock raised to maturity in a culture system are not yet available, although considerable progress has been made. In the interim, wild broodstock will have to be used. The method we have described here permits year-round production of larval lobsters from broodstock obtained through the commercial fishery without the usual legal or social complications.

REFERENCES

Aiken, D. E., and S. L. Waddy. 1976. Controlling growth and reproduction in the American lobster. Proc. World. Maricult. Soc. 7: 415-430.

1980a. Maturity and reproduction in the American lobster, p. 59-71. In Can. Tech. Rep. Fish. Aquat. Sci. 932.

1980b. Reproductive Biology, p 215-276. In The Biology and Management of Lobsters (J. S. Cobb and B. F. Phillips, eds.), Vol. 1, Chapt. 4, Academic Press.

1980c. Brood stock control in a lobster culture system. ICES C.M. 1980/F:30.

1980d. Methods for estimating size at maturity of male Homarus americanus. CAFSAC Res. Doc. 80/45, 10 p.

1982. Cement gland development, ovary maturation and reproductive cycles of female American lobsters, Homarus americanus. J. Crust. Biol. 2: 315-327.

Aiken, D. E., S. L. Waddy, K. Moreland, and S. M. Polar. 1984. Electroejaculation and artificial insemination of the American lobster, Homarus americanus. J. Crust. Biol. 4: (in press).

Aiken, D. E., S. L. Waddy, L. S. Uhazy, and A. Campbell. 1983. A nemertean destructive to the eggs of the lobster, Homarus americanus. Rapp. P.-v. Réun. Cons. Int. Explor. Mer 182: 130-133.

Dunham, P. J., and D. Skinner-Jacobs. 1978. Intermolt mating in the lobster (Homarus americanus). Mar. Behav. Physiol. 5: 209-214.

Fisher, W. S., E. H. Nilson, J. F. Steenbergen, and D. F. Lightner. 1978. Microbial diseases of cultured lobsters. A review. Aquaculture 14: 115-140.

Harper, R. E., and P. Talbot. 1984. Analysis of the epibiotic bacteria of lobster (Homarus) eggs and their influence on the loss of eggs from the pleopods. Aquaculture 36: 9-26.

Hedgecock, D. 1983. Maturation and spawning of the American lobster, Homarus americanus, p. 261-290. In CRC Handbook of Mariculture, Vol. 1, Crustacean Aquaculture (J. P. McVey, ed.).

Hedgecock, D., W. L. Moffet, W. Borgeson, and K. Nelson. 1978. Progress and problems in lobster broodstock development. Proc. World Maricult. Soc. 9: 497-506.

Hughes, J. T., R. A. Shleser, and G. Tchobanoglous. 1974. A rearing tank for lobster larvae and other aquatic species. Prog. Fish. Cult. 36: 129-132.

Krouse, J. S. 1973. Maturity, sex ratio and size composition of a natural population of American lobster, Homarus americanus, along the Maine coast. Fish. Bull. 71: 165-173.

Nelson, K., D. Hedgecock, and W. Borgeson. 1983. Photoperiodic and ecdysial control of vitellogenesis in lobsters (Homarus) (Decapoda,Nephropidae). Can. J. Fish. Aquat. Sci. 40: 940-947.

Perkins, H. C. 1971. Egg loss during incubation from offshore northern lobsters (Decapoda: Homaridae). Fish. Bull. 69: 451-453.

1972. Developmental rates at various temperatures of embryos of the northern lobster Homarus americanus Milne-Edwards. Fish. Bull. 70: 95-99.

Richards, P. R., and J. F. Wickins. 1979. Lobster culture research. Lab. Leafl., MAFF Direct. Fish. Res., Lowestoft, No. 47, 33 p.

Rosemark, R., and D. E. Conklin. 1983. Lobster pathology and treatments, p. 371-377. In CRC Handbook of Mariculture, Vol. 1, Crustacean Aquaculture (J.P. McVey, ed.).

Schuur, A, W. S. Fisher, J. C. Van Olst, J. M. Carlberg, J. T. Hughes, R. A. Schlesser, and R. F. Ford. 1976. Hatchery methods for the production of juvenile lobsters (Homarus americanus). Univ. California Sea Grant Publication 48, 21 p.

Sindermann, C. J. (ed.). 1977. Disease diagnosis and control in North American marine aquaculture. Dev. Aquaculture Fish. Sci. 6: 151-183.

Talbot, P., C. Thaler, and P. Wilson. 1984. Spawning, egg attachment and egg retention in captive lobsters (Homarus americanus). Aquaculture 37: 239-249.

Van Olst, J. C., J. M. Carlberg, and J. T. Hughes. 1980. Aquaculture, p. 333-390. In The Biology and Management of Lobsters (J. S. Cobb and B. F. Phillips, eds.), Vol. 2, Chapt. 10, Academic Press.

Waddy, S. L., and D. E. Aiken. 1981. Natural reproductive cycles of female American lobsters (Homarus americanus), p. 353. In Developments in Endocrinology, Vol. 11, Advances in Invertebrate Reproduction, Elsevier/North-Holland.

1984. Environmental regulation of ovarian development in the American lobster, p. 649. In Advances in Invertebrate Reproduction (W. Engels, W. H. Clark Jr., A. Fischer, P. J. W. Olive and D. F. Went, eds.), Elsevier.