ORGAN INDICES AND BIOCHEMICAL LEVELS OF OVA FROM PENAEID SHRIMP MAINTAINED IN CAPTIVITY VERSUS THOSE CAPTURED IN THE WILD

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ABSTRACT

This paper characterizes further penaeid shrimp reproduction by reporting: (1) the gonad, hepatopancreas, tail and eyestalk indices for unablated and unilateral ablated penaeid shrimp (P. setiferus) maintained in captivity versus shrimp captured in the wild; and (2) the biochemical content of spawned ova from three species of penaeid shrimp (P. setiferus, P. stylirostris and P. vannamei) obtained from wild populations and one species (P. setiferus) matured and spawned in captivity. The data indicated that (1) the dietary and environmental regimen used in this study is not optimum for reproduction of P. setiferus in captivity; (2) the hepatopancreas but not the tail muscle is directly involved in penaeid reproduction; (3) the population of P. setiferus sampled on June 2 and July 22, 1978, in the northwestern Gulf of Mexico was not at its reproductive peak; (4) the percent protein, lipid and carbohydrate content of unhatched eggs of P. setiferus, P. stylirostris and P. vannamei are very similar; and (5) biochemical analysis of the unhatched eggs and the determination of organ indices during reproduction are important parameters to obtain for understanding and evaluating penaeid reproduction in the wild and in captivity.

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INTRODUCTION

Shrimp aquaculture on a commercial basis is at the threshold of reality. However, there is obviously need and opportunity for tremendous improvement in both the efficiency and predictability of all phases of the present procedures used for penaeid shrimp aquaculture. The most limiting and critical of these phases is seed-stock availability. The best solution for the problem of seed-stock availability is an efficient and predictable system for penaeid shrimp reproduction in captivity. A procedure which will provide seed-stock by penaeid shrimp reproduction in captivity in quantities with a cost and predictability sufficient to support a commercial penaeid shrimp operation has not been reported.

Various degrees of success of penaeid reproduction in captivity have been reported for several species (Penaeus setiferus, Johnson and Fielding, 1956; Penaeus duorarum, Iyogi, 1971; P. duorarum, Caillouet, 1972; Penaeus orientalis, Penaeus monodon, Armstrong and Beard, 1975; Penaeus azteces, P. setiferus, Duronset et al., 1975; Penaeus californiensis, Moore et al., 1974; Penaeus merguiensis, Penaeus japonicas, P. azteces, Metapenaeus ensis, Aquacop, 1975; P. japonicus, Laubier-Bonichon and Laubier, 1976; P. setiferus, Penaeus stylirostris, Comte, 1977; P. stylirostris, P. setiferus, Comte et al., 1977; P. merguiensis, P. azteces, P. japonicas, P. monodon, M. ensis, Michel, 1977; P. monodon, Santiago, 1977; P. Japonicas, Laubier-Bonichon, 1976; P. setiferus, Brown et al., 1979). Some aspects of the reproductive biology of penaeid shrimp have been reported for several species (P. setiferus, Heegard, 1953; P. setiferus, Lindner and Anderson, 1956; P. duorarum, Sidred, 1958; P. duorarum, Cummings, 1961; Metapenaeus dobsoni, Metapenaeus affinis, Penaud indicus, Parapeneaopsis stylifera, Rao, 1968; P. setiferus, Lindner and Cook, 1970; M. affinis, Pilley and Nair, 1971; Penaeus semisulcatus, Metapenaeus stebbingi, Trachypenaeus granulosus, Leduc, 1977). Cytological and histological characterization of penaeid shrimp maturation and gametogenesis of both laboratory matured and wild penaeid shrimp also have been reported (P. japonicas, Huddinaga, 1942; P. duorarum, Caillouet, 1972; Lu et al., 1973; P. azteces, P. setiferus, Duronset et al., 1975). However, weight and biochemical changes of the organs involved in reproduction and of the ova for penaeid shrimp have been reported only in one publication describing the seasonal gonad indices for M. affinis (Pilley and Nair, 1971) and one publication of biochemical changes in P. setiferus (Middleditch et al., 1979). The determination of weight and biochemical changes of organs involved in reproduction and of ova for many marine invertebrate species has greatly increased the understanding of reproduction in marine invertebrates (see reviews Gleiss, 1959, 1966a, 1969; Gleiss and Pearse, 1974; Lawrence, 1976). Thus, the purpose of this paper is to characterize further penaeid shrimp reproduction by reporting: (1) the gonad, hepatopancreas, tail muscle and eyestalk indices for unablated and unilaterally ablated penaeid shrimp (P. setiferus) maintained in captivity versus shrimp captured in the wild, and (2) the biochemical content of spawned ova from one species (P. setiferus) matured in captivity and three species (P. setiferus, P. stylirostris and Penaeus vannamei) obtained from wild populations.

MATERIALS AND METHODS

Animals were obtained from wild populations of P. setiferus from the northern Gulf of Mexico on June 2 and 12 and July 22, 1978, and of P. stylirostris and P. vannamei from the Gulf of Panama from January through June 1978. Populations of P. setiferus maturing and spawning in captivity were sampled on September 4, 1978. The procedure used for the maturation and spawning of these animals in captivity has been described (Brown et al., 1979).

All animals obtained from the wild were sacrificed within 18 hours of capture. The animals to be sacrificed were weighed, headed and the ovary or testes, hepatopancreas, eyestalks and eyes (only one if from a unilaterally ablated animal), and tail muscle removed, weighed and frozen immediately for biochemical analysis.

Spawned eggs (ova) from animals matured in captivity and from mature females (P. setiferus) from the wild were sampled within 12 hours of the time of spawning. After the number of eggs was estimated by aliquot sampling, the eggs were separated on a nylon mesh and then frozen immediately. After partial dehydration (at least 75% of the water present in the eggs is removed) of the eggs in a frostless freezer, the eggs were completely dried under vacuum in a desiccator at room temperature (22-24°C). Appropriate amounts, 3-7 mg, 3-7 mg, and 75-200 mg, of the dried eggs were used for the determination of the percent protein, carbohydrate and lipid respectively. The protein content of the eggs was determined using the colorimetric method of Lowry et al. (1951). The total carbohydrate content was estimated by boiling the dried eggs in 5% trichloroacetic acid, centrifuging, and testing the supernatant with the procedure of Dubois et al. (1956). The total lipid determination was made by extracting the lipid from the dried eggs and weighing the extracted lipid according to the method of Freeman et al. (1957). All chemicals used were reagent grade.

Data are reported in organ indices which are organ wet weights (in grams) divided by wet whole body weight (in grams) times 100 and percent protein, lipid and carbohydrate which are weight of protein, lipid and carbohydrate (in grams) respectively in the sample divided by dry weight of sample (in grams) times 100. Significance of differences was determined using the two tailed student's t test.

RESULTS

There were no significant differences in the percent protein, carbohydrate and lipid of eggs of the three species of penaeid shrimp obtained from wild populations (Table 1). The ranges of percent protein, carbohydrate and lipid for the three species were 27.3-42.3, 2.9-3.1 and 16.1-20.2 respectively. The percent protein and carbohydrate contents of ova from P. setiferus matured in the wild versus captivity are very similar. The percent lipid content of the ova from animals matured in captivity appears to be higher than the percent lipid level of the ova from animals matured in the wild. Further data are required to determine if this difference is significant.
Table 1. Biochemical Content of Ova from Three Penaeid Species Captured in the Wild and from One Penaeid Species Matured in Captivity*

<table>
<thead>
<tr>
<th>Species</th>
<th>Source</th>
<th>Protein</th>
<th>Carbohydrate</th>
<th>Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. vannamei</td>
<td>wild</td>
<td>42.0 ± 3.0a</td>
<td>3.1 ± 0.2a</td>
<td>16.7 ± 1.5a</td>
</tr>
<tr>
<td>P. stylirostris</td>
<td>wild</td>
<td>37.3 ± 1.0a</td>
<td>3.0 ± 0.1a</td>
<td>16.1 ± 1.8a</td>
</tr>
<tr>
<td>P. setiferus</td>
<td>wild</td>
<td>42.3 ± 1.5a</td>
<td>2.9 ± 0.3a</td>
<td>20.3 ± 2.5a</td>
</tr>
<tr>
<td>P. setiferus</td>
<td>cap.</td>
<td>40.1</td>
<td>3.1</td>
<td>26.5</td>
</tr>
</tbody>
</table>

*Values (% dry weight) represent means ± S.E.M. of the number of observations in parentheses (each sample contained 10^5 to 3 x 10^6 eggs as estimated by weighing). Females P. vannamei and P. stylirostris were collected from the Gulf of Panama from January through June 1978. Female P. setiferus was obtained from the northern Gulf of Mexico on June 2 and July 22, 1978.

aProtein, carbohydrate and lipid values of ova from the three species of animals from the wild are not significantly different (p > 0.05).

The original average body weights of the males and females used for the mortality and laboratory experiments were 31 g and 35 g, respectively. After the animals were maintained in the laboratory for 74 days on the dietary regimen and environmental conditions previously described (Brown et al., 1979), the mean body weights for the unablated males and females were 35.1 and 47.7 g respectively and for the unilaterally ablated males and females, 32.9 and 44.9 g respectively (Table 2) of the animals maintained in the laboratory. The females were much more than the males. The mean wet body weight of each sex for the animals maintained in the laboratory at the time of their sacrifice was 7.9 g less than the mean body weight of animals of the same sex from the wild.

There are no significant differences in the mean tail muscle indices whether one compares wild to laboratory animals, male to female, or unablated to unilaterally ablated animals (Table 2).

The hepatopancreas indices were larger for the females than for the males for all three groups (i.e., wild, unablated captive and unilaterally ablated captive) with only the unilaterally ablated female hepatopancreas index not being significantly larger than unilaterally ablated male hepatopancreas index (Table 2). This lack of significance may be due to the small number of observations for the two groups. All the mean hepatopancreas indices of the males and females maintained in the laboratory were significantly larger than the respective indices of males and females from the wild except for the unilaterally ablated males versus the males from the wild. There were no significant differences between the hepatopancreas indices of the laboratory maintained unablated males or females as compared to the unilaterally ablated males or females.

The mean eyestalk index, representing the ratio of the weight of the two eyestalks and eyes to whole body weight, of the unablated females and males is not twice the mean eyestalk index, representing the ratio of the weight of one eyestalk and eye to the whole body weight, of the unilaterally ablated females and males respectively (Table 2). This may indicate that the remaining eyestalk and eye of the unilaterally ablated shrimp enlarges in response to the loss of one eyestalk and eye due to unilateral ablation.

Table 2. Body Weight and Hepatopancreas, Eyestalk, and Tail Muscle Indices of Shrimp (P. setiferus) from the Wild and from Unilaterally Ablated and Unablated Shrimp Matured in Captivity*

<table>
<thead>
<tr>
<th>Site</th>
<th>Eye</th>
<th>Sex</th>
<th>Obs.</th>
<th>Body Weight</th>
<th>Tail</th>
<th>Hepatopancreas</th>
<th>Eyestalk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild unablated</td>
<td></td>
<td></td>
<td>(52)</td>
<td>37.4 ± 0.8</td>
<td>1.62 ± 0.08</td>
<td>0.95 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Wild unablated</td>
<td></td>
<td></td>
<td>(36)</td>
<td>36.1 ± 1.2</td>
<td>1.49 ± 0.12</td>
<td>0.92 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Capture unablated</td>
<td></td>
<td></td>
<td>(16)</td>
<td>39.8 ± 1.7</td>
<td>1.04 ± 0.16</td>
<td>0.97 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Capture unablated</td>
<td></td>
<td></td>
<td>(14)</td>
<td>36.5 ± 1.9</td>
<td>1.43 ± 0.11</td>
<td>1.13 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Capture unilateral</td>
<td></td>
<td></td>
<td>(10)</td>
<td>30.0 ± 1.3</td>
<td>1.35 ± 0.42</td>
<td>0.70 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Capture unilateral</td>
<td></td>
<td></td>
<td>(3)</td>
<td>33.2 ± 1.5</td>
<td>4.56 ± 0.45</td>
<td>0.94 ± 0.03</td>
<td></td>
</tr>
</tbody>
</table>

*Values represent means ± S.E.M. of the indicated number of observations in parentheses. Values for the indices and body weight are wet organ weight ± wet body weight x 100 and grams respectively. Half of the wild animals were collected on June 2 and half on July 22, 1978. All the animals maintained in the laboratory were collected on June 12 and sacrificed on September 23, 1978. All animals were collected from the northern Gulf of Mexico.

Two eyestalks and eyes were used for the determination of these values.

Tail values of wild versus captive or male versus female are not significantly different (p > 0.05).

Hepatopancreas values of females significantly greater than those for males (p < 0.05).

Hepatopancreas values of animals from wild significantly less than unablated animals maintained in captivity (p < 0.05).

There are no significant differences between the mean gonad index of the females and males from the wild versus the females and males respectively which were maintained in the laboratory (Table 3). However, the high individual gonad index for a female from the wild group is considerably larger than the high individual gonad index for the unablated and unilaterally ablated females maintained in captivity. Conversely, the low individual gonad index for a female from the wild group is smaller than the low individual gonad index for the unablated and unilaterally ablated females maintained in captivity. Also, the mean gonad index for the females was significantly larger than the mean gonad index for the males for two (wild and unablated laboratory) of the three groups. Again, the lack of significance between the female and male gonad index of the unilaterally ablated group may be due to the small sample size.
Table 3. Gonad Indices of Shrimp (P. setiferus) from the Wild and Unilaterally Ablated and Unablated Shrimp Maintained in Captivity

<table>
<thead>
<tr>
<th>Site</th>
<th>Eye</th>
<th>Sex</th>
<th>Obs.</th>
<th>Mean ±SEM</th>
<th>High</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild</td>
<td>unablated</td>
<td>?</td>
<td>52</td>
<td>5.07 ± 0.47&lt;sup&gt;a&lt;/sup&gt;,&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.00</td>
<td>0.53</td>
</tr>
<tr>
<td>Wild</td>
<td>unablated</td>
<td>♂</td>
<td>25</td>
<td>3.12 ± 0.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.73</td>
<td>1.25</td>
</tr>
<tr>
<td>Captive</td>
<td>unablated</td>
<td>?</td>
<td>26</td>
<td>5.20 ± 0.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.66</td>
<td>1.86</td>
</tr>
<tr>
<td>Captive</td>
<td>unablated</td>
<td>♂</td>
<td>14</td>
<td>3.04 ± 0.22</td>
<td>4.00</td>
<td>0.27</td>
</tr>
<tr>
<td>Captive</td>
<td>unilateral</td>
<td>♂</td>
<td>10</td>
<td>4.48 ± 1.03</td>
<td>11.78</td>
<td>1.62</td>
</tr>
<tr>
<td>Captive</td>
<td>unilateral</td>
<td>♂</td>
<td>3</td>
<td>4.20 ± 1.43</td>
<td>5.63</td>
<td>2.77</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values represent means ±S.E.M. of the indicated number of observations in parentheses. Values for the indices are wet organ weight x wet body weight x 100. Each of wild animals were collected on June 2 and half on July 22, 1978. All animals maintained in the laboratory were collected on June 12 and sacrificed on September 23, 1978. All animals were collected from the northern Gulf of Mexico.

<sup>b</sup>Female gonad indices significantly greater than male gonad indices (p<0.05).

<sup>c</sup>Female gonad indices of all three groups are not significantly different (p>0.05).

<sup>d</sup>Male gonad indices of all three groups are not significantly different (p>0.05).

DISCUSSION

There are no reports in the literature concerning the percentage of protein, carbohydrate and lipid levels for unhatched penaeid ova. The similarity of the biochemical constitutions of ova from three penaeid shrimp species suggests that the percentage of protein, carbohydrate and lipid content of the eggs of the genus Penaeus might be very similar. Pandalus (1970, 1972) reported 43% and 49% lipid for the unhatched ova of the lobster (Homarus gammarus) and isopod (Liocarcinus) respectively as compared to 16.7%, 16.1% and 20.2% for the unhatched ova of the penaeid species reported in this study. However, the percentages of protein and carbohydrate in unhatched ova of the lobster, isopod, and penaeid shrimp were similar.

Gonad, hepatopancreas, eyestalk and tail muscle indices were determined for animals from wild populations in order to establish a baseline which was used to evaluate the maturation of the same species (P. setiferus) in captivity. Gonad indices have been used to study marine invertebrate reproduction in many species (Glase and Pearse, 1974; Lawrence, 1976). Seasonal gonad indices were used to study reproduction of three decapod crustaceans (Uca annulipes, Portunus pelagicus, Metapenaeus affinis) (Pillay and Nair, 1971). They obtained high mean gonad indices of 11.73 and 1.11 and low mean gonad indices of 1.36 and 0.56 for females and males respectively for the penaeid shrimp (M. affinis). They observed a high individual gonad index of 14.09 for one female.

The mean gonad index (5.07) obtained for females from the wild in this study indicates that the P. setiferus sampled on June 2 and July 22, 1978, were not at their reproductive peak although obviously there was reproduction occurring in the wild as indicated by the high individual gonad indices for some wild females. In contrast, June and July have been reported as the peak spawning months for P. setiferus in the northern Gulf of Mexico (Lindner and Anderson, 1955; Lindner and Cook, 1970). The high individual gonad index of 15.0 for P. setiferus in close to the high individual gonad index for M. affinis (14.09). Decapod crustaceans with a definite annual reproductive cycle can have different peak reproductive times even for succeeding years (Pillay and Nair, 1971). The dietary regimen and/or environmental conditions used in this study (Brown et al., 1979) were not optimum, even though maturation and spawning were occurring, because the mean gonad index of the females matured in captivity was less than the high mean gonad index (about 14) during the time of peak reproductive activity reported in other decapod crustaceans (Pillay and Nair, 1971). Further, the high individual gonad index for a female matured in captivity is less than the high individual gonad index for female decapod crustaceans in the wild (this study and Pillay and Nair, 1971). The seasonality of gonadal development has been associated with storage of energy in the hepatopancreas with a concomitant transfer (e.g., lipid) to the gonad during gametogenesis for asteroids, amphipodians and crustaceans (Glase, 1966a,b; 1969; Adiyodi and Adiyodi, 1974; Lawrence, 1976). Specifically, Adiyodi and Adiyodi (1971) reported that for the crab, Paranthusa biplicatos, there is a large increase in the lipid content of the ovary with a simultaneous decrease in the lipid content of the hepatopancreas at the end of the first phase of vitellogensis (stages 2-3). Quayry et al. (1974) also reported that the ovary and hepatopancreas are the main lipid storage organs and that there is an increased lipid content in the ovary during sexual development. They indicated that there may be a mobilization of lipids in the hepatopancreas in relation to vitellogenic requirements in a penaeid shrimp (P. japonicus). The significantly larger hepatopancreas indices for females than males in this study also suggest an involvement of the hepatopancreas in penaeid maturation. The higher hepatopancreas indices of the female population maturing in captivity versus wild, even though the mean gonad indices are not different, suggest the possibility of a lack of mobilization of nutrient reserves from the hepatopancreas to the ovary due either to an inadequate diet (qualitatively and/or quantitatively) or environmental regimen. In any case, hepatopancreas indices of captive versus wild animals also suggest that the procedure used for this study (see Brown et al., 1979) is not optimum for penaeid reproduction.

Hormones play a very important role in crustacean reproduction (Adiyodi and Adiyodi, 1970, 1974). Further, eyestalk ablation is either necessary or enhances maturation and spawning in captive and wild penaeid species (Avise et al., 1972; Avise et al., 1975). A compensation in terms of increase in size of the remaining eyestalk and eye relative to body weight for the unilaterally ablated may be indicated by the eyestalk indices observed in this study. No significant differences were seen for the gonad, hepatopancreas and tail muscle indices for each sex for unilaterally ablated versus unilaterally ablated females. Data on gonad growth production from the unilaterally ablated females as compared to unilaterally ablated females were reported by Brown et al. (1979) for P. setiferus.
The tail muscle indices were determined since the tail represents such a large percentage of the biomass of the penaeid shrimp. Tail muscle also served as a control organ. There is no reported mobilization of nutrient reserves from the tail muscle to ovary during penaeid maturation. The lack of significant differences between the tail muscle indices reported in this paper for any group of shrimp suggests that components of tail muscle are not directly mobilized for penaeid reproduction.

In conclusion, hepatopancreas, tail muscle and eyestalk indices and biochemistry of the eggs provided a more complete characterization and evaluation of penaeid shrimp reproduction. These data indicated participation of the hepatopancreas in penaeid reproduction and that the procedure used in this study was not optimum for penaeid shrimp reproduction.

SUMMARY

The data presented in this paper indicate that: (1) the dietary and environmental regimens used in this study is not optimum for reproduction of P. setiferus in captivity; (2) the hepatopancreas but not the tail is directly involved in penaeid reproduction; (3) the population of P. setiferus sampled on June 22 and July 23, 1976, in the northeastern Gulf of Mexico was not at its reproductive peak; (4) the percent protein, lipid and carbohydrate content of unattached eggs of P. setiferus, P. stylirostris and P. hansenii are very similar; and (5) biochemical analysis of the unattached eggs and the determination of organ indices during reproduction are important parameters to obtain for understanding and evaluating penaeid reproduction in the wild and in captivity.

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LITERATURE CITED


