ACHIEVING ADVANCED MATURATION AND SPAWNING IN YELLOWTAIL *SERIOLA QUINQUERADIATA* BY THE MANIPULATION OF PHOTOPERIOD AND WATER TEMPERATURE

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**ABSTRACT**

Yellowtail *Seriola quinqueradiata* held in captivity begin maturing and spawning in late April to early May, while yellowtail in the wild spawn about 2 mo earlier. The 2 mo difference in spawning periods is significant, as the cultivated fry are smaller than those in the wild when they are released for stock enhancement. The purpose of this study was to develop techniques for obtaining eggs of yellowtail in captivity earlier than usual to coincide with the natural spawning season of yellowtail in the wild. The first experiments examined the effects of extended daylength (1800-2400; EDL) on ovarian maturation and the use of human chorionic gonadotropin (HCG) to induce final maturation and spawning. In 1991, 1992, and 1993, female broodstock were induced to mature more rapidly than those under natural photoperiod by extending daylength by 6 h for 28 ds (1991, 1992) or 20 ds (1993). Each year, the mean number of eggs from fish under EDL was higher than that of the control group. The results demonstrate that manipulation of daylength is an effective method for accelerating maturation in female yellowtail broodstock.

Further experiments in 1994-1995 and 1995-1996 examined the combined effects of photoperiod control on the maturation of yellowtail, namely, a short-day (SD) treatment of 1 mo followed by a long-day (LD) treatment of 1 mo. In both years, the daylength was set to 8 h (8 L: 16 D) for 1 mo followed by a 10 h extension (18 L: 6 D) for the next mo under controlled water temperature. Female broodstock kept under controlled photoperiod and water temperature were induced to mature more rapidly than those maintained under natural conditions. However, neither controlled photoperiod nor warm temperature alone was sufficient to induce maturation in yellowtail. After HCG injection, during both years, fish kept under EDL or warm temperature spawned earlier than usual in captivity. Consequently, photoperiod and water temperature manipulations are effective in accelerating maturation of female yellowtail broodstock to the point where fertilized egg production can be achieved by the induction of spawning using HCG.

**INTRODUCTION**

The wild population of yellowtail *Seriola quinqueradiata* is one of the most valuable fishery resources in Japan. However, the natural stock has steadily declined over the years. In 1978, the first program for broodstock management and production of the juveniles of yellowtail for stock enhancement was initiated by the Japan Sea-Farming Association (JASFA) to offset the decline. Due to the advancements in techniques for the induced spawning of broodstock and the rearing of larvae and juveniles, as many as 1 million juveniles/yr have been produced.

The spawning of wild yellowtail in the waters around Shikoku and Kyushu has been observed from late February to April (Umeda 1991). However, the spawning season of yellowtail reared in net cages under natural conditions at the JASFA Komame Station in Kochi Prefecture occurs about 2 mo later. Due to this delay in spawning in captivity, artificially-produced juveniles are much smaller than wild juveniles at the time of release, resulting in poorer survival. The project initiated by JASFA was designed to obtain eggs at an earlier period in order to release tagged juveniles close to the same size and same age as juveniles found in the wild.

There have been many investigations of controlling the natural spawning season in fish by manipulating environmental factors such as photoperiod and water temperature (Breton and Billard 1977; MacQuarrie et al. 1978; Whitehead et al. 1978; Beacham and Murray 1993). The present study focused on determining appropriate photoperiod and temperature regimens to result
in the production of spawned eggs at an earlier time than usual from captive yellowtail broodstock.

MATERIALS AND METHODS

Yellowtail Broodstock

Experiment 1 was conducted during 1991 and 1993. Yellowtail used as broodstock in 1991 (Table 1) were captured by set-nets in Komame inlet (Kochi Pref.) and reared on moist pellets (MP) (Mushiake et al. 1993) for about 2 yr in a floating rectangular net cage (10 x 5 x 6 m) at the Komame Station of JASFA. All females used for the experiments were marked individually by a personal identification tag (PIT) (Identification Devices Inc., USA), implanted into the dorsal muscle when they were transferred from the net cage into indoor spawning tanks (110 m³). Fish used in the 1992 and 1993 experiments were transferred from a private farm in Ehime Prefecture to the Komame Station and fed on moist pellets for 1.5 yr as captive broodstock.

In experiment 2, during 1994-1995 and 1995-1996, the adult yellowtail were captured by set-nets in the Komame inlet and fed moist pellets (MP) or commercial soft-dry pellets (SDP: Sakamoto Fish Feed Co. Ltd., Chiba, Japan) (Mushiake et al. 1995) during 1994 and 1995, respectively, at Komame Station (Table 1). The fish were reared in net cages (10 x 10 x 6 m) for a period of 8 mo and transferred to indoor spawning tanks (65 m³) for spawning on 14 November in 1994 and 1995. All females were marked individually by PIT.

Experimental Rearing Conditions

Table 1 shows a summary of the two different groups in experiment 1 and the four groups in experiment 2. Each group in experiment 1, either the EDL-treated or control, consisted of 20 (1991) or about 10 (1992, 1993) individuals. The experiments were begun by placing the fish in the spawning tanks (110 m³). All fish were kept under natural lighting conditions from sunrise to 1800. The group exposed to EDL received

<table>
<thead>
<tr>
<th>Year</th>
<th>Group No.</th>
<th>Origin (M:F)</th>
<th>No. of fish</th>
<th>Diet¹</th>
<th>Fork length ±SD (cm)</th>
<th>Body weight ±SD (kg)</th>
<th>Condition factor ±SD</th>
<th>Rearing condition</th>
<th>Photoperiod²</th>
<th>Water temperature³</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991</td>
<td>Wild-1 20</td>
<td>12:8 MP</td>
<td>82.5±3.3 11.38±1.53 20.22±1.21</td>
<td>controlled-1</td>
<td>natural</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1992</td>
<td>Wild-2 20</td>
<td>12:8 MP</td>
<td>82.4±2.9 11.42±1.60 20.24±1.34</td>
<td>controlled-1</td>
<td>natural</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1993</td>
<td>Wild-2 20</td>
<td>12:8 MP</td>
<td>77.8±2.3 9.31±0.83 19.79±1.36</td>
<td>controlled-1</td>
<td>natural</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1995</td>
<td>Wild-1 6</td>
<td>3:3 MP</td>
<td>81.9±1.7 10.36±1.24 18.65±1.05</td>
<td>controlled-2</td>
<td>controlled</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1996</td>
<td>Wild-1 6</td>
<td>3:3 MP</td>
<td>81.6±1.8 10.14±1.46 18.67±1.15</td>
<td>controlled-2</td>
<td>controlled</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Wild-1: Captured by set-net fishery and reared for 2 years, Wild-2: Captured at juvenile stage and reared for 4 years in all.
² M: male; F: female.
³ MP: Moist pellets (formulas feed prescribed by National Research Institute of Aquaculture, Japan + raw fish (1:1)); SDP: Commercial soft dry pellets (Sakamoto Fish Feed Co. Ltd., Chiba, Japan).
⁴ Controlled-1: Extended daylength treatment (EDL) from 18:00 to 24:00, controlled-2: short-day treatment for one month followed by EDL treatment for the next one month.
⁵ Controlled: Water temperature was kept at a minimum of 19 C.
additional lighting provided by two tungsten flood lights (200 W/l) supported above each tank from 1800 to 2400. The EDL treatment was initiated on the day the fish were stocked into each experimental aquarium, and continued for 28 (1991, 1992) or 20 (1993) days at which time they were injected with human chorionic gonadotropin (HCG) to induce final maturation and spawning. The water temperature in experiment 1 was maintained around 19°C for the duration of the experiment.

In experiment 2, the broodstock in group 1 were maintained under both controlled photoperiod and water temperature. In group 2 the photoperiod and in group 3 the water temperature was controlled, respectively. No environmental manipulation was provided for fish in group 4. Photoperiod manipulation consisted of a short-day (SD) treatment followed by EDL treatments. The SD treatment was performed by spreading a matted black sheet (light transmittency 0%) over the surface of each tank from 1700 in the evening until 0900 the next morning (8 L-16 D). The SD treatment was initiated on d 3 (17 November; d 0 in Fig. 2) after transferring the fish into the indoor spawning tank, and continued for either 31 d (until 18 December 1994) or 32 d (until 19 December 1995). The EDL treatment commenced on the day following the termination of the SD treatment and continued for either 32 d (until 19 January 1995) or 34 d (until 22 January 1996). The EDL treatment was attained by extending the daylength 1 h every 3 or 4 d until daylength reached 18 h. The long photoperiod (18 L) was maintained from 19 January 1995 and 22 January 1996, throughout the spawning period.

The water temperature of the temperature-controlled tanks (1 and 3) in 1994-1995 and also 1995-1996 was kept at a minimum of 19°C by inflow of sea water heated with thermostatic devices. In groups 2 and 4, the water temperature was allowed to fluctuate naturally.

**Induced Spawning by Hormone Injection and Evaluation of Egg Quality**

In experiment 1, in order to induce spontaneous spawning in the indoor spawning tanks, HCG was injected at a dosage of 600 IU/kg BW into the dorsal muscle of both sexes, on d 28 (31 March 1991; 4 April 1992), or d 20 (25 March 1993) of EDL treatment.

In experiment 2, HCG was injected on d 76 in 1995 (1 February) and d 97 in 1996 (19 February). About 2 d after the administration of HCG in both experiments, fish began to spawn. Eggs were collected each day from 1700 to 0900 for as long as the fish continued to spawn. The number of eggs produced per fish/d was estimated by counting the number of eggs in a volume of 1 ml.

The diameters of 50 buoyant eggs, percent fertilization and the number of eggs having more than one oil droplet (abnormal eggs) were examined using a profile projector (Nikon). Percent hatching was also determined by estimating the numbers of larvae in each net. The percentage of normal larvae was estimated by counting the deformed and abnormal larvae (larvae having more than one oil droplet or having unusual oil deposition).
RESULTS

Ovarian Maturation by the EDL Treatment

The changes in oocyte diameters with time in all test groups in experiment 1 (1991-1993) and 2 (1994-1995, 1995-1996), are summarized in Fig. 1 and 2, respectively. Mean oocyte diameters at the start of experiment 1 ranged between 562 and 584 µm as shown in Fig. 1. In 1991 and 1992, mean oocyte diameters in the EDL group on d 28 were significantly (P<0.01) larger than those in the control groups. In 1993, the oocyte diameters in the EDL group on d 20 were also significantly (P<0.01) larger than those obtained from the control group.

In experiment 2, conducted in 1994-1995 and 1995-1996 (Fig. 2) the mean oocyte diameters at the beginning of experiments ranged from 309 to 314 µm and 339 to 347 µm, respectively. In 1994-1995, the mean oocyte diameters in the group 4 (that did not receive any environmental control) were 343 µm on d 31, 438 µm on d 63, and 504 µm on d 76 of the experiment. Mean oocyte diameters in fish from the photoperiod and water temperature-controlled group (1) were 373, 503, and 735 µm on d 31, d 63, and d 76, respectively. The mean oocyte diameters of fish from group 1 on d 76 was significantly larger (P<0.01) than those obtained from group 4. The mean oocyte diameters of females from either the photoperiod (2) or water temperature (3) controlled groups were intermediate to those of groups 1 and 4. In 1995-1996, the mean oocyte diameters were 359, 436, and 511 µm in group 4 and 393, 542, and 784 µm in group 1 on d 32, d 66, and d 97, respectively, indicating a significant difference (P<0.01) in the state of maturation between the two groups. The trend of increasing mean oocyte diameters in the other two groups (2 and 3) was similar in both years.

Induced Spawning

The results of induced spawning trials of yellowtail broodstock injected with HCG in experiment 1 and 2, are summarized in Tables 2 and 3, respectively. In experiment 1, the numbers of eggs produced in the EDL treatment during 1991 and 1993 were significantly (P<0.01) higher.
Table 2. Induced spawning results of yellowtail injected with HCG in experiment 1

<table>
<thead>
<tr>
<th>Year</th>
<th>Test group</th>
<th>Spawning period</th>
<th>Spawning days</th>
<th>EDL*</th>
<th>Control</th>
<th>EDL*</th>
<th>Control</th>
<th>EDL*</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Apr.3-Apr.15</td>
<td>13</td>
<td></td>
<td>Apr.3-Apr.17</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Apr.7-Apr.16</td>
<td>10</td>
<td></td>
<td>Apr.7-Apr.16</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mar.28-Apr.9</td>
<td>13</td>
<td></td>
<td>Mar.28-Apr.9</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


**Significantly different (p<0.01) as compared with the result of the control in the same year (t-test).

\*Significantly different (p<0.05) as compared with the result of the control in the same year (t-test).

Table 3. Induced spawning results of yellowtail injected with HCG in experiment 2

<table>
<thead>
<tr>
<th>Year</th>
<th>Test group</th>
<th>1994 - 1995</th>
<th>1995 - 1996</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. 1</td>
<td>No. 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Feb.3-Feb.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>


**Significantly different (p<0.01) in the number of normal larvae obtained between the two groups.

In experiment 2, only the fish in test group 1 spawned on d 2 after administration of HCG in 1994-1995 and in 1995-1996. In 1994-1995, after the injection of HCG, the broodstock began to spawn on 3 February and spawned daily until 9 February. They produced $418.0 \times 10^6$ eggs per fish, 37.2% of which were buoyant. In 1995-1996 the fish injected with HCG began to spawn on 21 February, and spawned daily from 24 February to 3 March. They produced $1541.7 \times 10^6$ eggs/fish, 49.2% of which were buoyant.

**DISCUSSION**

Changes in the mean oocyte diameter with time in experiment 1 (Fig.1) clearly indicate that ovarian maturation was accelerated by the EDL treatment. All data concerning egg quality indicated that the broodstock exposed to EDL treatment produced eggs that were superior to those in the control groups. This result demonstrates that the maturity of yellowtail broodstock can be manipulated by EDL to result in large numbers of eggs of suitable quality at a time that should result in the production of seedlings of appropriate size for use in stock enhancement activities.
As shown in Fig. 2, changes in mean oocyte diameters of broodstock in experiment 2 indicate that ovarian maturation in yellowtail was accelerated by manipulating both photoperiod and water temperature. The broodstock of all groups were injected with HCG on 1 February (on d 76) and 19 February (on d 97) in 1994-1995 and 1995-1996, respectively. The fish having mean oocyte diameters around 700 μm could be spawned by HCG injection, although the quantity and quality (percent buoyancy, fertilization, and hatching) were low. HCG-treated fish that possessed mean oocyte diameters around 800 μm (1995-1996) responded by spawning a larger number of eggs that were decidedly of better quality. Therefore, it was concluded that the success of accelerated egg production from yellowtail broodstock would also depend on administering HCG at the appropriate state (mean oocyte diameter of 800 μm) of maturity.

Spawning results (number of eggs and egg quality) of group 1 in 1995-1996 were superior to those in 1994-1995, but not as good as those reported previously from other culture activities (Mushiake et al. 1995). However, for the stock enhancement of yellowtail, in order to release juveniles of similar size to those in the wild, egg production earlier than the normal captive spawning period is required. Further research is necessary to improve the egg quality of from the advanced spawning yellowtail when maintained under controlled photoperiod and water temperature conditions.

For pink salmon Oncorhynchus gorbuscha (Beacham and Murray 1988, 1990), it has been suggested that acceleration of maturation is more likely to be achieved through manipulation of photoperiod rather than water temperature. The distinction between which environmental parameter is more important could not be discerned for the yellowtail broodstock, as the mean oocyte diameters from (photoperiod-controlled) and (water temperature-controlled) did not differ significantly from each other.

Although the mechanism by which ovarian maturation is accelerated remains to be clarified, it was found that yellowtail kept under controlled photoperiod and water temperature were able to spawn in February, 2 mo earlier than those held under ambient conditions. These results indicate that yellowtail juveniles can be produced earlier than usual at the JASFA Yushima Station, Kagawa Prefecture. In 1996, seed production was conducted and tagged juveniles were released into the sea 2 mo earlier than previously reported. The released fish, which were similar in size to their counterparts in the wild, showed a high percent recovery (12.9%) compared with the usual 0.2–3.1% of fish produced and released during the usual time near the eastern part of the Seto Inland Sea (unpublished data).

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LITERATURE CITED


THE APPLICATION OF DEEP SEA WATER IN JAPAN

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ABSTRACT

Deep sea water (from a depth of more than 200 m) has cold temperature, abundant nutrients, and good water quality that is pathogen-free and stable. Basic research on the utilization of this water for fisheries in Japan began in 1976 and at present, deep-seawater pumping systems are established in Toyama and Kochi Prefectures and under construction in Shizuoka and Okinawa Prefectures. The research emphasis of many national organizations, prefectures, universities, and private companies is shifting from basic research to feasibility studies or practical applications of deep sea water. For example, in Kochi Prefecture, located in southern Japan, it was found that deep sea water is advantageous in the aquaculture of cold-water species. Current fisheries-related projects include:

- aquaculture (sea vegetables, fishes, shellfish, etc.)
- basic research on deep sea organisms
- restoration of sea grass habitats

A wide range of projects unrelated to fisheries that are utilizing deep sea water to develop new industries and contribute to local economies include:

- the food industry
- medical treatment facilities
- cooling water for power stations
- agriculture of cold climate vegetables

Future investigations should focus on further explorations of deep sea water attributes, a cascade system for using deep sea water, reduction of costs, and potential environmental impacts.

INTRODUCTION

Currently, the utilization of deep sea water (DSW) is receiving much attention due to its high productivity, large quantity, and potential for recycling energy. Deep sea water, accounting for 95% of all sea water, generally refers to sea water from a depth of more than 200 m. DSW circles the globe over a period of about 2000 yr, and the up-welling of DSW occurs regularly in the oceans and seas throughout the world. Although it constitutes no more than 0.5% of all water in the seas, up-welled deep sea water is highly productive, supporting nearly 50% of all sea products. With the worldwide population explosion contributing to an ever-increasing consumption of animal protein, the focus on aquaculture will undoubtedly also intensify. The high productivity of DSW as a renewable energy source may increase the role of aquaculture to cultivate food for the expanding human population.

History of Research on the Uses of Deep Sea Water

Research on the applications of DSW is occurring worldwide, particularly in Hawai‘i, USA, at the Natural Energy Laboratory of Hawai‘i Authority (NELHA), and in Norway. The focus in Japan on the research and developmental studies for further applications of deep sea water forms the basis of this paper.

The history of research efforts in Japan on the applications of DSW is depicted in Table 1 (Nakajima 1998). In 1976, basic research efforts were begun by the Agency for Science and Technology. Between 1976 and 1986, we conceptualized deep sea water, developed an understanding of the characteristics of deep sea water, and developed the technology for the pumping of DSW. From 1986, the agency funded a 5-yr research program which resulted in the establishment of deep-seawater pumping systems in Kochi and Toyama Prefectures.
Table 1. History of research.

1976 Basic research
1986 Granted by the science and technology agency
1989 Water pumping systems in Kochi and Toyama Pref.
1997 Exploration of practical applications

Water Pumping Systems in Japan

In 1989, a buoyant catenary system of pumping water was installed in Toyama Bay, Toyama Prefecture, to examine whether on-site productivity could be increased by mixing deep sea water with surface water. That system was replaced by an above-ground system in 1994. In Kochi Prefecture, the first above-ground system of water pumping was installed in 1989 and the second in 1995. Some of the intake water has been shared with private companies, which have designed many products around the use of deep sea water. In Okinawa Prefecture, private companies jointly installed a buoyant catenary system called “Umi-Yakara 1-gou” (Fujii 1998), bringing the number of locations with DSW pumping systems in Japan to three (Fig. 1).

In 1997, egg production of cold-water organisms such as Japanese flounder was accomplished in Kochi Prefecture using DSW (Okamura and Doi 1998). This led to the shift from basic research to feasibility studies and further exploration of practical applications.

The quantity of DSW intake is 920 t/d in Kochi Prefecture and 3000 t/d in Toyama Prefecture (Table 2). The depth of water intake is about 300 m and water temperature is 9.5 °C in Kochi and 2 °C in Toyama Prefecture. As of yet, there are no large systems in Japan with the capacity or economic efficiency of the system at NELHA in Hawai’i, which pumps water from the deep sea at 88000 t/d (Hachmuth 1991).

Research in Toyama and Kochi Prefectures indicates many practical possibilities for future applications of DSW and currently many requests for the water are from private companies which have many projects in the planning stages along the coasts of Japan (Table 2). In Kochi Prefecture, a third DSW intake pipe was installed early this year. This system will provide 4000 t/d, 2000 t of which will be utilized for fisheries, with the remainder of sea water provided to companies such as cosmetic firms and chemical companies. In Okinawa Prefecture, a large system is planned which will provide 15 000 t/d of deep sea water (Shimoji and Torinaga 1997). This system will service research facilities as well as a resort. In Shizuoka, plans are being made for the intake of deep sea water from two different sources, one originating from the Kuroshio Current and the other originating from

<table>
<thead>
<tr>
<th>System</th>
<th>Capacity (m³/day)</th>
<th>Intake depth (m)</th>
<th>Length from shore (m)</th>
<th>Temp. (degrees C)</th>
<th>Date Installed</th>
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<tbody>
<tr>
<td>Kochi 1</td>
<td>460</td>
<td>320</td>
<td>2,650</td>
<td>9.5</td>
<td>1989</td>
</tr>
<tr>
<td>Kochi 2</td>
<td>460</td>
<td>344</td>
<td>2,650</td>
<td>9.5</td>
<td>1994</td>
</tr>
<tr>
<td>Toyama</td>
<td>3,000</td>
<td>321</td>
<td>3,060</td>
<td>2</td>
<td>1995</td>
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<tr>
<td>Okinawa 1</td>
<td>-</td>
<td>600,1400</td>
<td>30,000</td>
<td>9,2,6</td>
<td>1997</td>
</tr>
<tr>
<td>Okinawa 2</td>
<td>15,000</td>
<td>600</td>
<td>-</td>
<td>-</td>
<td>2000</td>
</tr>
<tr>
<td>Kochi 3</td>
<td>4,000</td>
<td>300</td>
<td>2,074</td>
<td>-</td>
<td>2000</td>
</tr>
<tr>
<td>Shizuoka</td>
<td>3,000</td>
<td>350,700</td>
<td>-</td>
<td>-</td>
<td>2001</td>
</tr>
</tbody>
</table>
the Oyashio Current.

MATERIALS AND METHODS

Fisheries Related Applications: Feasibility Studies

New ways of using deep sea water for fisheries, agriculture, energy, medical treatment, and environmental purposes are under constant investigation in Japan. Currently, the major use by fisheries is in the aquaculture of fishes, shellfish, sea vegetables, and phytoplankton. The fisheries' sector is also looking into the practical application of handling of captured fish with deep sea water to maintain freshness. Salinity and environmental restoration using the abundant nutrients of deep sea water is another avenue of research.

Aquaculture

A major advantage of using deep sea water for aquaculture is the ability to culture cold-water organisms and deep-ocean organisms in tropical areas. Another is the ease at which water temperature can be controlled by mixing surface water with deep sea water. A third advantage is disease control, as there are few viruses and pathogenic bacteria in deep sea water. A disadvantage of using surface sea water is the maintenance required to keep the water intake pipes free of organisms that cling to the pipes and foul the water. However, when DSW is used for aquaculture purposes, maintenance of the pipes to remove harmful bacteria and other organisms is not necessary. In the Kochi Prefectural Deep Seawater Laboratory, the intake pipes haven't required cleaning for the past 10 yr (Miyamoto 1999).

The species targeted for aquaculture in Japan are mainly those requiring cold, deep, ocean water (Table 3). Most of the aquaculture projects that rely on that type of water are carried out in Kochi and Toyama Prefectures (Fujita 1997; Taniguchi 1997). Almost all the projects are at experimental level, but the egg production of Japanese flounder has been demonstrated to be practical since 1997 (Okamura and Doi 1998).

The abundant nutrients in deep sea water have instigated many projects for the production of sea vegetables and micro algae. In Kochi Prefecture, they have succeeded in producing edible konbu, and cold water sea vegetables. The growth of konbu in pumped deep sea water is reportedly the same as in its natural habitat in Hokkaido Prefecture, in the northernmost section of Japan, which is known as the production center of konbu (Yamaguchi et al. 1994).

Table 3. Target organisms of aquaculture using deep seawater in Japan.

<table>
<thead>
<tr>
<th>Target organisms</th>
<th>Organizations</th>
</tr>
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<tbody>
<tr>
<td><strong>Fishes</strong></td>
<td></td>
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<tr>
<td>Japanese flounder</td>
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</tr>
<tr>
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<td>Kochi Pref.</td>
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<tr>
<td>Butterfish</td>
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<td>Toyama Pref.</td>
</tr>
<tr>
<td>Firefly squid</td>
<td>Toyama Pref.</td>
</tr>
<tr>
<td>Shrimp</td>
<td>Japan Sea-Farming Association, Okinawa Pref.</td>
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<tr>
<td>Whelk</td>
<td>Toyama Pref.</td>
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<tr>
<td>Oyster</td>
<td>Japan Marine Sci. &amp; Tech. Center</td>
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<td><strong>Vegetables</strong></td>
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<td>Japanese tangle</td>
<td>Kochi Pref., Toyama Pref.</td>
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<td>Wakame</td>
<td>Kochi Pref.</td>
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<td>Laver</td>
<td>Kochi Univ.</td>
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<td>Sea trumpet</td>
<td>Kochi Pref.</td>
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<tr>
<td>Microalgae</td>
<td>Kochi Pref., Toyama Pref., etc.</td>
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<tr>
<td>Precious coral</td>
<td>Kochi Pref.</td>
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<tr>
<td>Plankton</td>
<td>Kochi Pref., Toyama Pref.</td>
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</tbody>
</table>

Handling of Captured Fishes

In Japan, there are many instances when captured fish are not taken directly to market. One such instance is when fishermen hold them in port until prices increase. Therefore, it is necessary to keep the captured fishes sanitary and fresh until sold. Research for the application of DSW in the
handling of captured fishes is focused on the purity of the water. Studies include the use of DSW to wash captured fish in the fishing ports in order to keep them fresh and also to transport the fish. Frozen deep sea water, for example, has already been shown to be effective in the transporting of fish (Kawasaki and Kuyou 1998).

Environmental Restoration
Trials are underway to examine whether the abundant nutrients of deep sea water can be applicable to environmental restoration efforts. The loss of the sea grass habitat is an important topic in Japan. Many efforts have been made to restore sea grass habitats, but most have not been very successful thus far.

In Kochi Prefecture, however, the DSW is discharged into the near-shore ocean waters after it is used. As a result, sea grass was discovered growing along the coastal areas, even where it had not grown before. Accordingly, a new avenue of research developed recently in Kochi Prefecture (Taniguchi et al. 1998) using DSW to initiate restoration of the coastal habitat. In Toyama Prefecture, mixing DSW with surface water to raise on-site productivity was attempted, but it met with limited success as the quantity of DSW was low and the vertical mixing was difficult due to the heavier density of deep sea water (Iseki et al. 1994).

Applications Unrelated to Fisheries
Businesses unrelated to fisheries, such as the food industry, medical treatment facilities, utility companies, and agriculture have found the usage of DSW advantageous. Applications in the food and medical industries are now practical. The use of DSW to cool water for power stations and utilization for agriculture are being explored as future applications.

Food Industry
Various foods and beverages are being produced using desalinized or concentrated deep sea water. Products such as jelly, mineral water, soy sauce, Japanese sake, confectioneries, and salt are made in Kochi Prefecture, contributing to the local economy (Hisatake 1997). These products are very popular due to the “mellow” flavor associated with the water. The exact role of DSW in changing the flavor or taste, however, is not fully understood.

Medical Treatment
It is empirically known that sea water is effective for the treatment of atopic dermatitis. However, because of the many bacteria and viruses in surface sea water, it is not recommended for daily application. As an alternative, pure, deep sea water was tested for daily use with promising results (Nomura 1995). Other medical researchers are trying to extract valuable chemicals from micro algae grown in deep sea water (Matsunaga et al. 1997; Komai et al. 1997).

Agriculture
In the subtropical environment of Okinawa Prefecture, it is not possible to grow cold climate vegetables, such as spinach, during the summer months. However, when cold, DSW was pumped through fields of spinach in underground pipes, the spinach grew very well. Such applications of deep sea water for agriculture are performed at NELHA in Hawai‘i, USA, where they have succeeded in producing various cold-season vegetables and crops in the tropics (Daniel 1994).

The abundant nutrients of deep sea water are also favorable for agriculture. When watered with diluted deep sea water, spinach actually grew faster than when tap or surface water was used (http://www.lizard.co.jp/deep-sea/hourenso.html).
DISCUSSION

Future Applications

A variety of possible applications of deep sea water are topics of future research. One possible use is to cool the water emitted by power stations. Preliminary studies demonstrate that the cold temperature of DSW increases the efficiency of heat exchange. The small variation in temperature between discharged deep sea water and coastal waters reduces any potential damage to the near-shore environment. However, if deep sea water is to be used for cooling water at power stations, quantities as large as one million t/d would be needed. The intake of such a large quantity of DSW could affect the global environment, for example, by changing the balance of carbon dioxide or altering ocean currents. Therefore, environmental impact studies have been initiated.

Thalassotherapy, or medical treatment using sea water, is being examined. Currently, some resort facilities in Toyama Prefecture perform thalassotherapy using DSW (http://www.micnet.ne.jp/hotaru-n/museum/english/index.html).

Future uses of DSW in agriculture might be for hydroponic plant culture or maintenance of seed at cool temperatures.

Future Problems

Problems that might result from increased use of DSW are also under consideration. Although it is recognized that deep sea water is effective in various applications, the exact functions and ramifications are not clearly understood. Methods must be developed to reduce the high costs of constructing DSW water pumping systems. Further studies should be done to understand and assess the environmental impacts of intake and discharge of deep sea water on the coastal environment.

To alleviate some of the anticipated problems, a cascade system of using deep sea water has been proposed (Fig. 2, Ikeda 1997). In this system, intake water is used for air-conditioning and then used for aquaculture. After it is used for aquaculture, the DSW is discharged into the ocean for environmental restoration. This system can decrease the negative environmental impact of discharging cold water while maintaining the positive attributes of deep sea water. In Kochi Prefecture, DSW that has been used to culture sea vegetables then used in the aquaculture of abalone, is one method of using a cascade system whereby the deep sea water pumped from deep depths is utilized to the fullest and the environmental impacts are minimized.

Figure 2. Cascade system of using deep seawater.
ACKNOWLEDGMENTS

The authors greatly appreciate the assistance of the staff of the Kochi Prefectural Laboratory of Deep Sea Water and Dr. Kunio Takatsuki from Tokyo Kyoei, Inc., for providing valuable information.

LITERATURE CITED


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ACQUISITION AND LOSS OF POTENTIAL FOR MOTILITY OF SPERMATOZOA OF THE JAPANESE EEL ANGUILLA JAPONICA

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ABSTRACT

To date, cultivated male Japanese eels have not matured sexually in captivity under normal conditions. However, spermatogenesis and spermiation can be induced by the injection of gonadotropins. During this study, most males spermated after the fifth or sixth weekly injection of human chorionic gonadotropin (HCG; 1 IU/g BW/wk) and the milt weight gradually increased as the number of injections increased. Motility of spermatozoa (percent motility after dilution with 450 mM NaCl) from HCG injected males showed significant individual differences and periodical changes after each weekly injection of HCG. Motility of spermatozoa in Japanese eel milt was found to have an intimate relationship between the pH of the milt and concentration of potassium in the seminal plasma. Motility of spermatozoa could be regulated by changes in the ionic constituents of the isotonic incubation media before dilution with a hyperosmotic solution. The percent motility of spermatozoa in eel milt increased significantly after incubating for 60 min in isotonic artificial seminal plasma (ASP), which consists of NaCl + KCl + CaCl2 + MgCl2 + NaHCO3 buffered with TAPS-NaOH at pH 8.1, and with Ca2+, Mg2+ free-ASP. Motility, however, decreased rapidly in K+ free-ASP and in HCO3, free-ASP. These results indicate that acquisition and loss of the potential for motility of eel spermatozoa can be altered by changing the potassium and bicarbonate ion concentrations of the incubating medium irrespective of the initial potential for motility. The methods to obtain good quality spermatozoa described in this experiment should compensate for low volume of milt at the time of artificial fertilization.

INTRODUCTION

The freshwater Japanese eel (unagi) is one of the most widely cultivated species in Japan due to its popularity as a food fish. This popularity has caused a decrease in wild fry over the last 25 yr, as the eel fry under cultivation are wild-caught elver which have been captured in estuaries. The shortage of fry for cultivation has recently become a serious problem, leading to the intensive study of techniques for artificial breeding of the eel. In the present study, we investigated the factors which affect the milt quality in males artificially induced to mature, and examined the techniques for controlling the motility of eel spermatozoa.

INDUCTION OF TESTICULAR MATURATION BY HCG INJECTION

Cultivated male Japanese eels at 200-300 g BW are sexually immature and do not mature under normal culture conditions (Yamamoto et al. 1972). However, injection of gonadotropins can easily induce spermatogenesis and spermiation in the male for purposes of artificially propagating this species (Yamamoto et al. 1972). Chiba et al. (1997) reported that injections of human chorionic gonadotropin (HCG) administered to male eels less than 29 cm BL had no effect on the serum 11-ketotestosterone (11KT) levels, a major androgen in the eel (Miura et al. 1991), or on spermatogenesis. However, in fish more than 32 cm BL, testicular maturation was induced, accompanied by increases in plasma 11-KT levels. HCG has been used exclusively for the induction of sexual maturation in male Japanese eels, and a single injection of HCG at a relatively high dose (5 IU/g BW, Miura et al. 1991; 8 IU/g BW, Ohta and Tanaka 1997) is reportedly effective in inducing spermatogenesis.

To develop techniques for the artificial maturation of male eels, we investigated the number of weekly HCG injections required to obtain an adequate volume of high quality milt (Ohta et al. 1996a). Ten sexually immature males (252 g average BW) received 14 weekly injections of HCG (250 IU/eel/wk). Two out of 10 males spermated after the fifth injection, and most had
spermated after the sixth. The expressible milt weight gradually increased as the number of injections increased and became stable after injections 11-13. Percent motility of the spermatozoa measured after dilution with 450 mM NaCl increased after 7-9 injections and reached about 60-70% after the tenth injection. These results indicated that repeated weekly injections of HCG at 1 IU/g BW over 10 wk artificially induced sexual maturation in immature male eels.

PROBLEMS WITH INDUCTION OF TESTICULAR MATURATION

Although artificial induction of maturation in the immature male eel is not difficult, the milt obtained from an artificially matured male has not been sufficient in quantity or quality for high fertilization of eggs. First, the amount of obtainable milt from one male is small compared to the egg volume from one female. As already described, milt can be obtained from most of the males which received 10 or more weekly injections of HCG. The milt volume averages about 1 g (Ohta et al. 1996a), while the egg weight from one ovulated female often exceeds 300 g. Therefore, techniques must be developed to enable successful fertilization using the small quantity of milt.

Another problem is that individual differences in sperm motility are significant among the males induced to mature by artificial means. For example, Fig. 1 shows the percent motility of the milt from 10 randomly selected males which received 14 injections of HCG. Although the mean motility value was 55.3±8.2%, the lowest value was less than 10% and the highest more than 90%. It is clear that these differences in milt quality among males will lead to varying degrees of success in the fertilization of eggs. Furthermore, sperm motility changes with time after the administration of HCG. The changes in percent motility of spermatozoa in milt during the weekly injections of HCG are presented in Fig.2. The motility increased sharply 6 h after the injection and peaked after 24 h. Then, motility decreased by d 3 after injection, and remained at low level when tested on d 7. Milt obtained just prior to injection13 also showed low sperm motility. These periodic changes in percent motility will certainly affect the fertilization of eggs if the qualitative changes in milt quality at the time of artificial fertilization are disregarded. One possible solution to this problem is to give an additional injection of HCG 1 d prior to artificial fertilization (Ohta et al. 1997c).

RELATIONSHIP BETWEEN IONIC CHARACTERISTICS OF SEMINAL PLASMA AND SPERM MOTILITY

Changes in sperm motility observed during the weekly administration of HCG suggests

![Figure 1](image1.png)

Figure 1. Percentage of motile spermatozoa of the milt from ten randomly selected males when the milt was diluted at 1,000 times with 450 mM NaCl buffered with 20 mM HEPES-NaOH at pH 7.5. Spermatozoa were classed as motile when the sperm head showed forward movement at 15 sec after dilution when analyzed under the VTR-light microscope.

![Figure 2](image2.png)

Figure 2. Changes in sperm motility during weekly injections of HCG (1 IU/g BW/wk). A small amount of milt was obtained from each male (n=5) just prior to injection 13, and 6 h, 24 h, and 72 h after, and just prior to injection 14, and 24 h, 48 h, 72 h, and 96 h after.
that the increase or decrease of HCG concentration affects the aqueous environment surrounding the spermatozoa in the sperm duct. Therefore, the potential for changes in the motility of spermatozoa appear to be related to the time elapsed after administration of HCG. It is possible that the composition of the seminal plasma can also affect the motility of the eel spermatozoa.

The biochemical characteristics of seminal plasma in 109 male Japanese eels were investigated with reference to sperm motility. After measuring the pH of the milt, the percent motility of spermatozoa and the ionic concentration of the seminal plasma in the milt were measured, respectively. An intimate relationship was found between sperm motility, milt pH and potassium concentration in the seminal plasma. The mean milt pH was 8.05 ± 0.02 and the potassium concentration of the seminal plasma was 20.85 ± 0.52 mM (n=109). We classified the milt from the 109 males into four groups: 1) milt which showed both milt pH and potassium concentration of seminal plasma more than the mean values (n=29), 2) milt which showed milt pH more than the mean value and potassium concentration less than the mean value (n=12), 3) milt which showed milt pH less than the mean value and potassium concentration more than mean value (n=24), and 4) milt which had both pH and potassium concentration less than the mean values (n=44). The mean percent motility of spermatozoa in the milt of these four groups is shown in Fig. 3. Percent motility of spermatozoa in the milt, of which pH and potassium concentration of seminal plasma were above the means, were significantly (P<0.05) higher than samples with both values less than the means. Similar relationships could not be found for other cations (Na⁺, Ca²⁺, or Mg²⁺). These results indicate that the increase of potassium ions and decrease of protons in the seminal plasma stimulate the acquisition of sperm motility in the Japanese eel.

**ACQUISITION AND LOSS OF POTENTIAL FOR MOTILITY IN THE MILT SPERMATOZOA IN VITRO**

An artificial seminal plasma consisting of 149.3 mM NaCl + 15.2 mM KCl + 1.3 mM CaCl₂ + 1.6 mM MgCl₂ + 20 mM NaHCO₃ (buffered with 20 mM TAPS-NaOH at pH 8.1) was produced during the current study based on previous measurements of seminal plasma (Ohta et al. 1997a). Spermatozoa in milt was incubated in the ASP and changes in the potential for motility following dilution with 450 mM NaCl were recorded (Fig. 4). Before incubation, spermatozoa in the milt was observed at 53.4 ± 11.8% motility (initial control). The motility increased significantly when the milt was incubated with the ASP or Ca²⁺ and Mg²⁺ free ASP for 60 min (83.4 ± 2.5% and 86.1 ± 2.1%, respectively). In contrast, spermatozoa incubated with K⁺ free ASP or HCO₃⁻ free ASP showed a sharp decrease in motility within 30 min, and reached 1.8 ± 0.7 %

![Figure 3. Relationship between percent motility of the spermatozoa when diluted with 450 mM NaCl and pH of the milt, and concentration of potassium ions in the seminal plasma. Asterisks mean significant difference (P<0.05) between them.](image)

![Figure 4. Effects of cations or anions in the ASP on the acquisition and loss of sperm motility. Horizontal bars indicate means ± SEM of results from 10 eels.](image)
or 5.7 ± 2.2%, respectively. These results indicate that potassium and bicarbonate ions are essential for the acquisition and maintenance of motility of eel spermatozoa.

SOLUTIONS TO TECHNICAL PROBLEMS

The main problems with artificial induction of testicular maturation in the Japanese eel are: 1) the scarcity of milt compared with egg volume for successful fertilization, 2) the extent of individual differences in sperm motility among males artificially induced to mature, and 3) the change in motility with time after injections of HCG.

Low milt volume can be resolved by dilution of milt with appropriate diluent(s). Milt dilution has been reported to improve percent fertilization compared to undiluted milt which is low in volume (Poon and Johnson 1970; Billard et al. 1974; Rieniets and Millard 1987). Billard (1992) stressed the advantages of using diluents for artificial insemination compared to the dry method, and indicated the optimum sperm dilution of milt in salmonids is around 1000 times. In earlier studies of the Japanese eel, we reported the validity using artificial seminal plasma to dilute the milt up to 30 times (Ohta et al. 1997b) and 100 times (Ohta et al. 1996b; Kagawa et al. 1998) to improve percent fertilization.

Results from the present study suggests that the cause of the other two problems (individual differences and changes over time in sperm motility) is the lack of an effective regulatory mechanism of potassium and bicarbonate ions and/or proton concentrations in the seminal plasma of males treated with HCG. Although spermatogenesis can be readily induced by multiple injections of HCG, further improvement in hormonal treatment appears to be necessary for obtaining high quality milt.

A relationship between motility and the capacity for fertilization of teleost spermatozoa has been confirmed by several authors (Billard and Cosson 1992; Harvey and Kelley 1984; Ohta et al. 1995). The present study indicates that the potential for sperm motility in the Japanese eel can be regulated by incubating milt in an isotonic incubation media with a high concentration of potassium and bicarbonate ions. Techniques for obtaining good quality spermatozoa of the Japanese eel by the incubation method used in the current investigation should compensate for the shortage of milt at the time of artificial fertilization and improve percent fertilization.

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ENHANCED NUTRIENT SUPPLY TO NORWEGIAN COASTAL WATERS: EFFECTS ON GROWTH OF SCALLOPS AND BLUE MUSSELS

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ABSTRACT

An experiment including an enhanced nutrient supply to coastal waters was performed during 1996-1999 in a landlocked bay, Hopavågen, located in Central Norway. The aim of the study was to quantify effects of the nutrient enrichment on the food web structure and growth of blue mussels Mytilus edulis and scallops Pecten maximus.

INTRODUCTION

Hopavågen has a surface area of 27 ha, a total volume of 5.4 mill m³ and a mean depth of 20 m. The volume of euphotic waters is estimated to be 3.7 million m³, which corresponds to 68% of the total volume. Due to a narrow inlet, the tidal range in Hopavågen is limited to 0.3–1.0 m, compared to 0.8–2.3 m in the coastal waters outside of the bay. Daily water exchange in the bay averages 0.6±0.22 mill m³ or 0.11±0.04 % of total volume d⁻¹, corresponding to 19% of the productive waters d⁻¹.

MATERIALS AND METHODS

In the first 2 yr of the investigation, 1996 and 1997, physical, chemical and biological data were provided for an undisturbed situation. From early May to mid October 1998 and 1999 nutrients (phosphorus, silicate, and nitrogen) were added during the early tide period with inflowing water to the bay, corresponding to approximately two additions of nutrients daily. The addition of nutrients in 1998 corresponded to 0.4 µg phosphorus L⁻¹ d⁻¹ or an estimated 100% increase in the phosphorus supply to the bay. The molar ratio for N:Si:P was 15:5.4:1. In 1999 the addition of phosphorus was increased to 0.8 µg P L⁻¹ d⁻¹, and the molar ratio of N:Si:P was close to 16:8:1.

Data on growth of scallops and blue mussels are available for 1997 and for the first year nutrients were added, 1998. In the former year, scallops (40 mm) and blue mussels (45 mm) were placed in polyethylene baskets at three different depths and two different depths, respectively, in the central area of the bay. The growth of the shells was followed for 9 months (May 1997-February 1998). During the period June 1998–April 1999, the growth of scallops (25 mm) and blue mussels (40 mm) was followed at 2 and 10 m depth at four different sites in the bay and at a control station (Værnes) in a fjord about 1 km from the inlet of Hopavågen. Growth was measured as increase in shell height (SH). In 1998 the content of shell tissue (wet weight, dry weight and ash free weight) of blue mussels and scallops was measured at the end of the growth period.
RESULTS

Physical measurements revealed a 4-5°C higher temperature at 10 m depth in the late summer period (August and September) in 1997 (12°C) compared to 1998 (12°C). The temperature of the surface layer reached 20°C in early September 1997, compared to about 15°C in the following year. The salinity was in the range of 31–33% in both years, and the water current at 10 m at the four stations in Hopavågen varied from 1.1–1.8 cm s⁻¹.

The mean chlorophyll a content (June-September) was in 1996 and 1997 estimated to be 2.1 μg l⁻¹ and 1.8 μg l⁻¹, respectively. About 80% of the mean chlorophyll a content was in the fraction less than 20 μm in both years, and particles less than 2μm contributed 20% of the total chlorophyll a on average. The mean daily production for the period May-October in 1996 and 1997 was estimated to be 410 and 420 mg carbon m⁻² d⁻¹, respectively.

In the first year nutrients were added to the bay, the mean daily primary production increased to 580 mg carbon m⁻² d⁻¹. However, the mean chlorophyll a content, 2.0 μg l⁻¹, was at the same level as the previous year, and the chlorophyll a content, in different size fractions, did not reveal any change in size distribution of the phytoplankton following the nutrient additions. The bacteria biomass and production remained at the same level during the investigation.

The growth rate of blue mussels in the period July-September 1997 and 1998 was in the range of 0.14–0.29% d⁻¹ at the different depths and locations. The highest daily increase in SH was recorded in Hopavågen in 1998, but it was not significantly higher than in 1997 or at the control station at Værnes. In the late autumn period (September-October), the growth rates varied from 0 to 0.07% d⁻¹ at the sampling stations. The tissue content (wet and dry weight) at the end of the season was significantly higher in blue mussels in Hopavågen compared to mussels from the control station at Værnes.

The growth rate of scallops in Hopavågen increased from 0.16% d⁻¹ in the period July-September in 1997 to 0.53% d⁻¹ in 1998. During the latter year the recorded growth of SH of scallops in Hopavågen was significantly higher than the mean value for scallops grown in the fjord outside Hopavågen (0.44% d⁻¹). Also, between September and late October, the growth rate in the bay (about 0.20% d⁻¹) was much higher than in the previous year (0.04% d⁻¹). The tissue content (dry weight, ash free dry weight) in the scallops grown in Hopavågen was 2-4 times higher than in shells farmed at Værnes.

DISCUSSION

The addition of nutrients corresponding to an estimated annual supply of phosphorus and a molar ratio for N:Si:P of 15:5:4:1 caused a 46% increase in primary production in Hopavågen, compared to the pre-fertilization year. As the mean chlorophyll a content remained at the same level through the investigated period, the results indicate an increased turnover rate or increased grazing rate of the phytoplankton following the nutrient additions. The increased primary production in 1998 did not affect the SH growth of blue mussels Mytilus edulis. However, the shell content in mussels grown in Hopavågen was significantly higher than in mussels from the control station, which indicate that the increased production affected the somatic growth of the mussels. The SH of scallops Pecten maximus in the bay increased from 0.16% d⁻¹ in 1997 to 0.53% d⁻¹ in 1998. The recorded growth of scallops in Hopavågen in 1998 was also significantly higher than for scallops outside the bay (0.44% d⁻¹). Even more pronounced was a 2-4 times higher content of shell tissue in the scallops grown in Hopavågen in 1998, compared to individuals from the control station at Værnes.

The results reveal that nutrients may be considered an important resource also in management of aquatic systems. However, dose-response experiments in marine waters with different water qualities are needed to establish a general knowledge of effects of nutrient supply on the productivity of different aquatic organisms and the environmental impact in a long-term perspective.
SEED PRODUCTION TRIAL OF THE DEEP-SEA WHELK *BUCCINUM BAYANI* USING DEEP SEA WATER

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ABSTRACT

The edible deep-sea whelk *Buccinum bayani* is distributed at depths from 200-800 m in the Sea of Japan, and especially Toyama Bay. It is one of the most important commercial shellfish in Toyama Prefecture. In order to establish seed production and to obtain ecological information necessary for the management and stock enhancement of this species, indoor tank culture of the whelk was carried out at the Toyama Prefectural Fisheries Research Institute using running, deep sea water at a temperature of about 3 C that was pumped from a depth of 321 m. For preparation as broodstock, the whelks were cultured on a diet of fish such as sardine or mackerel and after 1 yr of culture, as many as 80% of the whelks survived and some of the females laid eggs. Most of the younger whelks that were initially < 60 mm in shell height (SH) grew, while the presumed older ones (up to 120 mm SH) showed very little growth. Histological examination, low variation in the gonad index (GI), and the recorded mo during which eggs were laid all strongly suggest that *B. bayani* has no definite reproductive season. Spawned eggs were deposited as a mass of 80 to 250 egg capsules; each capsule containing 1,500-3,500 eggs. Whelks with larger SH produced many more egg capsules with about 10-30 individuals hatching from one egg capsule 6 mo after being spawned. By feeding whelks the fish as mentioned previously, juvenile whelks grew well and 50% of them survived after 1 yr in culture.

INTRODUCTION

Toyama Prefectural Fisheries Research Institute has the capability to pump deep sea water (DSW) from Toyama Bay in the Sea of Japan at a depth of 321 m. The temperature of this DSW remains around 3 C year-round. The cold, clean, DSW is mainly used for stock enhancement or ecological studies on cold- and deepwater species such as Masu salmon *Onchorhyncus masou*, Pacific cod *Gadus macrocephalus*, red tanner crab *Chionocetes opilio elongatus* and Toyama shrimp *Pandalus hystsinotus*, as well as edible deep-sea whelks inhabiting the continental slope in the Sea of Japan. The whelks are among the most important commercial shellfish in Toyama Prefecture and are caught using chained basket traps. The catch of whelks in Toyama Bay (greater than 300 t) accounts for around 85% of the total catch of marine shells (Fig. 1). Because little biological information is available on the whelks, indoor culture trials of *Buccinum bayani* were carried out to establish mass-culture techniques and to promote ecological studies for the management and stock-enhancement of this important shellfish species.

![Figure 1. The total catches of marine shells and the catch of deep-sea whelks in Toyama Prefecture from 1977 to 1997. Solid line indicates the catch of deep-sea whelks and broken line indicates the total catches of marine shells.](image-url)
MATERIALS AND METHODS

Broodstock Culture

*B. bayani* of various sizes were collected for broodstock from Toyama Bay and cultured in 1-t tanks using running DSW under atmospheric pressure. Fish, such as sardine or mackerel, were given as food. Survival and growth, as determined by changes in shell height, were recorded over the course of 1 yr. When egg laying was observed, the mo of egg laying, SH of the egg-laying female and number of egg capsules were recorded.

Culture of Juveniles

The culture of juveniles was carried out in two 5-L tanks stocked with 100 juveniles/tank. The tank bottoms were covered with mud collected from the sea bottom at a depth of 200 m. To validate the hypothesis that juvenile *B. bayani* feeds on organic matter in the mud, fish meat such as sardines were given to the juveniles in one tank, while no food was given to those in the other tank. After 1 yr of culture, the survival and growth of juveniles were assessed.

Reproductive Cycle

*B. bayani*, more than 90 mm SH and presumed to be sexually mature, was sampled from Toyama Bay at 3 mo intervals. Fifteen to 20 shells were examined during each sampling and the gonad index (GI) was determined in the manner described by Takamaru and Fuji (1981); where GI is equal to gonad weight x 100/BW without the shell. The gonads were fixed in a formalin solution, dehydrated in alcohol, and embedded in paraffin wax in preparation for histological examination. Sections were cut at 8-12 μm, stained with Mayer's hematoxylin and counterstained with eosin.

RESULTS AND DISCUSSION

Broodstock Culture

After 1 yr in culture, as many as 80% of the whelks survived. Most of the younger cultured whelks that were less than 60 mm SH grew, while the older ones (up to 120 mm SH) rarely grew (Fig. 2). The reason for this difference is unknown, but perhaps a better food source must be investigated for older whelks to grow and mature.

During the course of culture, some of the females laid eggs (Fig. 3). Since egg laying was observed throughout the year (Fig. 4), it is suggested that *B. bayani* have no definite egg-laying season. During the egg-laying process, female *B. bayani* formed an 'egg mass' by producing 80-250 egg capsules (each capsule containing 1500-3500 eggs). A female, 73.5 mm

![Figure 2. Growth in shell height of Buccinum bayani after a year of culture. Growth = Shell height after a year of culture — Initial shell height.](image)

![Figure 3. Laying eggs of Buccinum bayani. Dome-shaped egg mass was comprised of egg capsules.](image)

![Figure 4. Number of female shell that laid eggs during indoor culture by months.](image)
SH, was the smallest whelk to lay eggs, and it was noted that the larger whelks laid more egg capsules. At the given temperature (3°C), it took 6 mo before the eggs hatched. About 10-30 individuals hatched from one egg capsule, while the other eggs in the capsule appeared to serve as nurse eggs. The shell height just after hatching was about 2 mm. As the females did not lay eggs again for at least 2 yr after previously laying eggs, further investigation is necessary to determine the life span and egg-laying cycles of whelks. Tag-and-release experiments may bring further information on the growth and survival rates of *B. bayani*, particularly in their natural habitats.

Culture of Juveniles

Juvenile *B. bayani* survived and grew on the mud substrate under both conditions; given fish meat and without any solid food (Table 1). Surviving and growing without any solid food for 1 yr strongly suggests that the juveniles could survive only on the organic matter in the mud. However, the growth of the whelks in the ‘mud group’ was inferior to that of the ‘mud + fish group’ where the weight of the ‘mud group’ was only one-sixth of the ‘mud + fish group.’ When given fish meat, the SH increased from 2.5 to 7.1 mm after 1 yr of culture and the survival was 51%. It appears that fish meat is a suitable food for juvenile as well as adult whelks.

Reproductive Cycle

The maximum gonad index value of the female whelks was about 12, which is approximately twice of that observed for males (Fig. 5). In both the males and the females examined, however, whelks that revealed a GI greater than half of each maximum GI value were reported in each mo throughout the yr. This was coincident with the above results that egg laying was observed year-round. Histological examination of testes and ovaries revealed the presence of mature spermatozoa and mature oocytes filled with large yolk granules (about 20 μm in diameter) in more than 80% of the males and more than 50% of the females, respectively, every representative mo of the four sampling periods (Fig. 6, 7). In addition, the year-round maturity in the males (>80%) suggests that there is no resting period in spermatogenesis. We are speculating that sperm is constantly produced in the testis and transported to the seminal vesicle.

![Graph](image)

**Figure 5.** Gonad index of *Buccinum bayani*. Gonad Index = gonad weight X 100/body weight without shell

<table>
<thead>
<tr>
<th>Table 1. Growth of juveniles of <em>Buccinum bayani</em> after a year of culture.</th>
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<tr>
<td><strong>Average</strong></td>
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<td>survival rate (%)</td>
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<td>Mud + fish meat group</td>
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<td>Mud group</td>
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<td><em>Initial juveniles</em></td>
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LITERATURE CITED


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