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Aquacultural ecology of hatchery-produced juvenile bay mussels, *Mytilus edulis* L.

Trevelyan, George Arthur, Ph.D.

University of California, Davis, 1991
Aquacultural Ecology of Hatchery-Produced Juvenile Bay
Mussels, *Mytilus edulis* L.

By

GEORGE ARTHUR TREVELYAN
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DISSERTATION

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in the

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Aquacultural Ecology of Hatchery-Produced Juvenile Bay Mussels, *Mytilus edulis* L.

Abstract

Hatcheries offer a realistic approach to solving many of the problems that constrain the mussel (*Mytilus edulis*) culture industry in the U.S. and elsewhere. But for this approach to be economically viable, growers need to be able to effectively use very small (1 mm), recently metamorphosed, and thus inexpensive spat. However, the ecology of these transplanted spat has received very little attention and previous attempts at using these spat have often failed. Therefore the purpose of this dissertation was to identify the major causes of mortality of recently planted spat and to analyze how management variables, such as planting season, initial density, and substratum type, affect production.

As a prerequisite to this study, a method was developed to reliably metamorphose *Mytilus edulis* larvae in the hatchery. This was achieved by using a downwelling system which is described. In addition, the spat so produced had to be tagged so that they could be distinguished from wild spat. A tagging method is described based on the finding that shell pigmentation was controlled by light intensity.
Field caging experiments using these tagged spat in Tomales Bay, CA showed that the seasonal pattern of inshore migration and reproduction of small surfperch, chiefly Cymatogaster aggregata, caused a seasonal pattern of high summertime mortality. This could be avoided by excluding the fish with netting (11 mm mesh diameter). A size refuge against these predators was reached at 11 mm. The flatworm, Notoplana inquieta, was also found to be an important predator. It could be eradicated using a 3 minute freshwater dip.

Mortality, fouling, and growth were found to be strongly density dependent. The highest yield was attained by planting at low density (2/cm²) in the month of March when fouling was light.

Large scale crops could be successfully started by remote setting of the spat in tanks of static, unaerated seawater at ambient temperature. In some cases, the seeded substrata could be removed from the tank after as little as 1.5 hr. Rubberized, curled hair packing material with a backing of scour pad was a good substratum, providing a protective, easily seeded habitat for the spat.
Aquacultural Ecology of Hatchery-Produced Juvenile Bay Mussels, *Mytilus edulis* L.

INTRODUCTION

1. Background and Rationale

A mussel farm can be thought of as an artificial reef that captures the primary production of phytoplankton from an extensive coastal area, converts it and concentrates it into a dense area of high secondary biomass production. For instance, the harvested biomass production of a 16 x 16 m mussel culture raft in Galicia, Spain typically ranges from 30,000 to 90,000 kg wet weight per year (Korringa, 1976). The creation and management of these "reefs" has become an important aquacultural activity in parts of the world.

In 1971, the world mussel harvest was 370,000 metric tons (MT) (Mason, 1976), but by 1986, this figure more than doubled, reaching 829,000 MT. This world mussel harvest for 1986 contributed 0.9% to the total world fisheries landings for that year (FAO, 1988). Nearly all of this mussel harvest was from some form of cultivation as opposed to fishing of wild stocks. Fifty percent of the world mussel production in 1986 came from the Atlantic coast of Europe, principally Spain, Denmark and the Netherlands. These countries have traditionally been the main world producers, but their importance has recently waned as China has dramatically increased production and in 1986 was the world leader, at
125,000 MT. Mussel production in the U.S., though comparatively small, has steadily increased over the last 2 decades and in 1986 reached 19,517 MT (2% of world total) (FAO, 1988). Most of the U.S. production came from Maine and Washington states (Lutz, 1985).

The principal species of mussel cultured is the bay or blue mussel, *Mytilus edulis*, which accounted for 53.8% of all mussel landings in 1986 (FAO, 1988). Of all invertebrate species landed in 1986, *M. edulis* was the third most important in terms of tonnage of whole animal, after the Pacific oyster, *Crassostrea gigas*, and the Antarctic krill, *Euphausia superba*. In addition to *M. edulis*, a variety of other mussel species are grown around the world. For instance, the closely related Mediterranean mussel, *Mytilus galloprovincialis*, was the second most important species of mussel landed in 1986, followed by the green mussel, *M. smaragdinus*, which is grown in Malaysia and Thailand.

In all nations around the world mussel farmers depend on natural recruitment for their seed supply. Mussel farming is thus a form of semi-culture in which the life cycle is not entirely controlled. Consequently, mussel farming has flourished only near those places where natural recruitment is consistently abundant. But in many locales, mussels recruit erratically or sparsely. This is the case along much of the Pacific coasts of the U.S. and Mexico, in bays such as South Puget Sound, Washington (C. Stevens, Kamilche
Sea Farms, pers. comm.), Winchester Bay, Oregon (R. Sardinia, Umpqua Aquaculture, Inc., Winchester Bay, OR, pers. comm.), Tomales Bay, California (pers. obs.), Todos Santos Bay, Baja California (L. Garcia Pamanes, Martesano Cultivos Marinos, Ensenada, Mexico, pers. comm.), and San Quintin Bay, Baja California (A. Aguirre, Bahia Falsa Cooperativa, Ensenada, Mexico, pers. comm.).

Even in places where mussel culture has developed, seed supply can be limiting. This is the situation in the Netherlands, where about 1 out of every 5 years there is not enough seed (Korringa, 1976). In New Zealand, much of the seed is collected from algal wrack washed up near the northern tip of the North Island and shipped to the growing areas in the South Island. This source of seedlings is unreliable (Hickman, 1987, 1989). Unavailability of seed is also a problem in India (Silas, 1980) and the Philippines (Anon., 1983). This widespread constraint to mussel culture could be alleviated through the use of molluscan bivalve hatcheries. By making mussel seed available on demand, hatcheries would allow both expansion of mussel farming to new areas and stabilization of production in established growing areas.

Hatcheries also offer a realistic approach to solving other difficult biological problems which constrain the industry. For instance, hemic neoplasia causes significant mortality to market size native mussels (Mytilus edulis) in Puget Sound and British Columbia (Skidmore and Chew, 1985).
Hatchery-reared seed from California broodstock were recently shipped to south Puget Sound and were grown alongside native mussels. Most of these native mussels died of this disease. In contrast, the introduced mussels did not become infected and showed no mortality to market size (C. Stevens, Kamilche Sea Farms, pers. comm.). Hatcheries could also be used to reduce predation. The most serious predators in mussel culture in North America are the scoter ducks, *Mellanitta* spp. There is evidence that the heavier shelled mussel, *Mytilus californianus*, is not eaten by these ducks (Behrens Yamada and Dunham, 1989). If hatcheries made available the spat of *M. californianus*, this species could be cultured in Oregon where market demand for this mussel is high and duck predation is severe (Behrens Yamada and Dunham, 1989).

Another difficulty in mussel culture is the sudden loss of a large percentage of the crop's biomass which occurs when the mussels spawn. This results in a poor, unsaleable product. Hatcheries could produce triploid mussels which will not spawn. This is being done now with oysters, *Crasostrea* spp. (Stanley et al., 1981; Allen et al., 1989). Alternatively, the Tomales Bay strain of bay mussel spawns in the winter and is in good condition in the summer when other strains, such as that found in Puget Sound, are spawned out (pers. obs.). Thus by controlling the whole life cycle, the culturist gains the capability to solve
problems, such as spawning, predation, and disease, and to thereby enhance production and quality.

There is a wealth of information available on the reproductive and larval biology of mussels, particularly of *Mytilus edulis*. The works of Bayne (1964, 1965, 1976), and Loosanoff and Davis (1963) are particularly valuable. Thus the procurement and rearing of the larvae is straightforward and could be easily done in commercial bivalve hatcheries.

Settlement and metamorphosis have also received some attention (Blok and Geelan, 1958; Bayne, 1965; Cooper, 1983; Trevelyan and Chang, 1983). Scaling this information up to large production levels can be a problem, as seen by Waterstrat *et al.* (1980). They found that survival through metamorphosis was often poor. But in general it is biologically possible to rear mussels through settlement. For instance, AQUACOP (1979) produced large numbers of green mussel, *Mytilus viridis*, post-larvae in French Polynesia. The post-larvae produced in hatcheries could then be safely reared in intensive nursery systems up to a size easily planted by growers, such as 10 to 15 mm in length, as is typically done with cultchless *Crassostrea gigas*. Thus in a strictly biological sense, economics aside, mussel seed can be produced with existing technology.

However, existing nurseries are certainly not producing these large, easily handled mussel seed. Probably part of the reason for this is that the economics of mussel farming do not justify such a large investment in the seed. Market
sized mussels do not fetch as high a price per individual in the market place as do Pacific oysters. For instance, in 1988 one could purchase cultured *Mytilus edulis* from Cove Mussels Co. on Tomales Bay, CA for approximately $0.05–0.10 each as opposed to approximately $0.35 each for *Crassostrea gigas*. Before hatcheries can integrate into mussel culture there has to be some innovation which brings the price of seed down.

One obvious strategy is to try to eliminate a long, intensive nursery phase and instead plant the spat into the sea when very small and inexpensive. If these spat could be reared with high survival, hatcheries might then become a viable component of mussel culture. This approach of planting small spat has received little attention and the few attempts were for the most part unsuccessful. Myers (1980) transplanted about 1 million small spat attached to ropes to his farm in Maine, but all spat were dead after 2 weeks. They apparently were smothered by rapidly growing algae. K. Chew (pers. comm.) and J. Richards (pers. comm.) also transplanted hatchery reared spat into Puget Sound, Washington. Both said that heavy losses of the spat occurred. The most successful transplantings were done in China. In the early 1970's, efforts were begun to produce mussel seed on a large hatchery scale in response to seed shortages (*Zhang et al.*, 1981; *Zhang et al.*, 1982; *Lou et al.*, 1982; Zhang, 1984). In 1974 for instance, 2.5 million seed mussels (2 cm in
length) were produced from small, hatchery-reared spat transplanted to the sea on palm fiber (coir) mats (Nie et al., 1979). It was found that spat transplanted when less than 3 mm in length disappeared from the substrata and that clear water and calm seas were favorable conditions for reducing the dislodgement of spat after transplantation. Survival rates were variable, but were 60% to harvest in one instance (Anon., 1977).

Thus, this work in China showed that spat could be transplanted successfully there. But it was not clear why spat smaller than 3 mm disappeared, or what were the rates and main causes of mortality and loss. Nor was it understood why the previous attempts here in North America failed, and how successful transplantings should be done. It would be economically much more favorable if the spat could be planted when they were less than 1 mm long, rather than 3 mm in length. However, no one has ever focused on the ecology of these small spat when transplanted into the sea.

2. Specific Questions Addressed

Therefore, the research in this dissertation addresses the following questions:

1. What are the mortality rates of transplanted spat?
2. What causes the mortality, and how does it vary with the season?
3. How does the initial density of mussel spat affect the degree of growth, fouling and mortality?
4. What practical materials and methods are successful for commercial scale production?

In order to answer these questions, it was necessary to be able to distinguish positively the planted spat from those settling naturally. In this way, the retention or survival rates could be measured unambiguously in the field. Unseeded control substrata were used in some cases to measure natural settlement, but this is not as good a control for natural settlement because settling larvae may be gregarious (Maas Geesteranus, 1942); the natural settlement onto seeded substrata would likely be greater than onto the unseeded controls. Therefore a way to tag the spat was needed. Chapter II describes the tagging of spat. The tagged spat were planted in the field while manipulating variables such as caging (Chapter III) and density (Chapter IV). Finally, several materials and methods for planting large crops were studied (Chapter V). In all these studies, growth and retention of the mussels on the substrata were measured.

3. Species Studied

Soot-Ryen (1955) lumped all the smooth-shelled Mytilus found along the west coast of North America into one species, *Mytilus edulis* L. This species embraced all *Mytilus* except *M. californianus* which is usually recognizable by its radiating ribs. But this lumping of all the *edulis*-like mussels into one species has recently been challenged by
MacDonald and Koehn (1988). Using protein electrophoresis, these authors found that there are 2 distinct forms of edulis-like mussels present on the west coast which are morphologically very similar. One of the forms is electrophoretically indistinguishable from M. galloprovincialis, the Mediterranean mussel, which is known to have recently colonized many disparate shores around the world. The other form is also distinct from the Atlantic M. edulis and was designated M. trossulus by MacDonald and Koehn (1988). In central and northern California, the galloprovincialis and trossulus forms co-occur and apparently hybridize with one another. These forms are very difficult to distinguish from one another morphologically, not only because they look alike, but because they both vary morphologically in response to their environment. There is as yet no morphological key for discriminating between them. Thus the species used in the present study is not easily defined.

MacDonald and Koehn (1988) had one sample from Tomales Bay which I collected for them from Marconi Cove. This cove was the source of most of the broodstock used in the present study. This sample proved to be pure Mytilus galloprovincialis. Thus M. galloprovincialis was probably the principal species under study. However, broodstock were also collected from many parts of Tomales Bay as well as from Bodega Harbor, CA and for one spawn, from an oil platform offshore from Santa Barbara, CA. It is quite likely therefore that both the trossulus and galloprovincialis forms,
and perhaps their hybrids, were used in the present study. It would be misleading then to consider this work to have been confined to _M. galloprovincialis_. Whether or not _M. galloprovincialis_ even deserves full species status is still being debated (Gosling, 1984). Whether or not _M. trossulus_ will ever be recognized as a distinct species is less clear still. Consequently, until this nomenclatural confusion is settled, the _edulis_-like mussels on the west coast of North America are still most clearly defined and embraced by the name, _Mytilus edulis_. While recognizing that they are a genetically diverse complex, the mussels studied in the present work will therefore be called _Mytilus edulis_.

The nomenclature for small, post-larval mussels is diverse. They may be called spat, post-larvae, plantigrades, seed or seedlings, or juveniles. Since growers usually refer to the 1-3 cm mussels as seed, this convention will be followed here. Below 1 cm, the mussels will be called spat.

4. **Study Site**

All the field work was performed at Marconi Cove in Tomales Bay, Marin County, California (Figure 1). Marconi Cove is a slight indentation on the east side of the bay, 13 km from the mouth. This cove was used because it is the site of a small commercial mussel farm, Cove Mussels Co., which provided access and water space and assistance for rearing the experimental crops of mussels. Mussels grown in
Figure 1. A. Map of Tomales Bay, California. The arrow approximately midway down the length of the bay shows the location of Marconi Cove. B. Map of California. The location of Tomales Bay is marked with an arrow.
this cove were known to grow rapidly, sometimes reaching 60 mm within 1 year (pers. obs.).

Three different stations within Marconi Cove were used. These were a dock, a raft, and a longline. The dock, located midway between the north and south points of the cove was situated in 1-2 m of water at mean lower low water (MLLW, 0 ft). The longline and raft were in 3 m of water at MLLW. The dock station was protected from the prevailing northwest winds while the other 2 stations were not. These 2 stations were frequently exposed to 0.3-0.5 m wind chop, particularly during the afternoon on spring and summer days. Occasionally during the winter, the dock was exposed to southerly gales which caused sections of the dock to break off. Sea surface temperatures in Marconi Cove varied between 8.4°C in February, and 21.9°C in August between July 1985 and September 1987 (see Chapter III, Figure 16). Salinity was not measured, but data are available for a nearby location (2 km to the north) (Smith et al., 1971). At that location, surface salinity remained high (28-34 ppt) between April and November, but between December and March salinities were much more variable, plummeting briefly to as low as 5 ppt after a rain.

5. Hatchery Production of Mussel Spat

A. Spawning and Fertilization

As mentioned above, broodstock mussels were collected primarily from Tomales Bay, as well as from Bodega Harbor, and on one occasion, from a Santa Barbara oil platform. No
conditioning of the broodstock prior to spawning was performed. Spawning was induced by heat shock as follows: The mussels were removed from ambient sea water (10-16°C) and were placed individually into battery jars containing 2 liters of 28-30°C sea water. Since these jars were placed in a room held at 18°C, the water in the battery jars slowly equilibrated over several hours to this temperature. Spawning began between 5 minutes and 2 hours after immersion in the warm sea water, but on average began after about 30 minutes. Between 30 and 100 mussels were used in any spawn attempt, and if 10% spawned, it was considered a success. Since the timing of the reproductive cycle was not known, spawning attempts were made in all months of the year (except December and January). Twenty six spawning attempts were made using Tomales Bay broodstock over a 3 year period. The best time of year for successful spawning was October through April, when 7 out of 8 attempts were successful. May and June were the poorest months for obtaining gametes, since none of the 6 attempts succeeded, and the late summer, July through September, was also poor, with just 3 out of 12 attempts succeeding.

The spawned eggs were collected and cleaned by screening and decanting. They were fertilized by adding sperm gradually while repeatedly examining the egg-sperm mixture microscopically. Sperm was added until approximately 2-10 sperm could be seen attached to each egg. After 10 minutes
of mixing, the eggs were allowed to settle to the bottom of the beaker, the excess sperm were decanted and the zygotes were transferred to the larval rearing tanks.

B. Larval Rearing

These tanks were 208 l (55 gallon) polyethylene tanks (Industrial Plastics, Lima, Ohio). The seawater used was sand filtered, then 1 μm filtered and U.V. irradiated. Salinity was 33-35 ppt. Every 3 days, the water was changed. The temperature of the fresh seawater was between 10 and 16°C, but this water warmed up to room temperature (18-20°C) over a 24 hour period. The larvae were fed Tahitian strain Isochrysis galbana (T-ISO) 1-2 times per day as needed. At each feeding, the algal density was brought up to approximately 125 cells/μl. Because the larvae did well with this simple monoalgal diet, no other algal food species were used. Larval density was maintained between 1 and 10 per ml.

C. Settlement and Metamorphosis

At 16-18 days post-fertilization, the pediveligers began clumping together, forming sticky strands that floated on the surface or adhered to the air rod (Figure 2). These clumps may have been caused by the larva's secretion of a thread or "trailing byssus" which became entangled with those of its neighbors. Cranfield (1973a) found that crawling pediveliger larvae of the flat oyster, Ostrea edulis, left behind a fine thread attached to the substratum which could be detected by lifting it off the bottom with a brush.
Figure 2. A clump of pediveliger larvae of *Mytilus edulis*
removed from the surface of a larval rearing tank.
The secretions from several different glands in the foot produced this thread (Cranfield, 1973b). The clumping phenomenon in *Mytilus* larvae has not yet been described and needs further study. This phenomenon clearly signalled their competency for metamorphosis, because as soon as they began clumping, they could be induced to metamorphose.

For the first batches reared in 1985, metamorphosis was encouraged simply by adding natural filamentous substrata, such as the hydroid, *Obelia* sp., and the red alga, *Polysiphonia pacifica*, to the tank. But this method was unsatisfactory because the larvae often accumulated in piles in "dead spots" (areas of poor circulation) on the bottom, underneath and away from the settlement substrata. There on the bottom they were susceptible to high mortality. Consequently, a conical tank (Figure 3) with no dead spots was used to keep the larvae in suspension. Larvae that sank to the bottom were air-lifted back to the surface and into a downwelling chamber with a 1 mm screen bottom containing filamentous red algae, usually *Polysiphonia pacifica*. I collected this alga, as well as other delicate, filamentous reds from under the floating docks of nearby marinas. The quantity of algae used was just sufficient to loosely fill the downwelling chamber. I inspected the algae microscopically prior to use. Bivalve larvae or post-larvae were never seen in the algae. An effort was made to use algae
Figure 3. Diagram of the conical downwelling tank used for settlement of mussel larvae. The chamber at the water surface has a 1 mm mesh screen bottom and is loosely filled with a filamentous red alga, such as *Polysiphonia pacifica*. Approximately 1 million 18 day old pediveliger larvae are added to the system.
Air Lift

ALGAE

400 liter

Figure 3
that were relatively free of gammarid and caprellid amphipods, which were sometimes abundant.

The cone was stocked with 1-3 million pediveliger larvae. A stocking level of 1 million larvae per 400 l cone tank is recommended since at this density crowding problems were minimized. Within 4 hours after stocking the tank, settlement was apparent on the algae as well as on the screen at the bottom of the downwelling chamber. After 2 days, nearly all the larvae had accumulated on the algae or on the chamber (Figure 4). At this time, the rate of consumption of T-ISO increased greatly.

New post-larval shell growth, signalling completion of metamorphosis, was apparent 23 days after fertilization. On or near day 24, the spat (500 μm mean length) were extracted from the algae. This was done by breaking up the algae and rolling it gently between the fingers and shaking it underwater. Another method was to use a seawater jet and screens to force the spat out of the algae. This procedure resulted in a mass of post-larvae which also contained a small amount of algae (Figure 5). At this time, the number of spat produced was estimated by extrapolation as described in Chapter V. Seven batches of larvae were metamorphosed using the downwelling cones over the course of this study. These batches yielded between 170,000 and 2,400,000 (mean=950,000) spat per cone. This was a mean yield of 2.4 spat/ml of cone volume.
Figure 4. Recently settled *Mytilus edulis* pediveligers and post-larvae, ranging in length between 260-360 μm, on a filamentous red alga held in a down-welling chamber. The measurement bar is 1.0 mm in length.
Figure 5. A mass of *Mytilus edulis* post-larvae, 400-700 μm in length, after extraction from the filamentous red algae upon which they settled and metamorphosed. Some fragments of the alga are still visible in the mass of post-larvae. The measurement bar is 5.0 mm in length.
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II. LIGHT-INDUCED SHELL PIGMENTATION IN POST-LARVAE
AND ITS USE AS A BIOLOGICAL TAG

INTRODUCTION

Young post-larvae of marine invertebrates are typically very small and inconspicuous, making measurements of mortality rates and movements difficult. Consequently, little is known of their ecology and behavior in the field. The development of molluscan hatchery technology, however, has made it possible to obtain large numbers of newly metamorphosed post-larvae of known species. These post-larvae can be transplanted and manipulated in different field situations. This "seeding" approach can facilitate investigations of post-larval ecology in natural or cultivated ecosystems.

To keep track of cohorts seeded in a habitat, tagging is often necessary. A number of methods have been developed to tag molluscan post-larvae. Dey and Bolton (1978) showed that the American oyster, *Crassostrea virginica*, and the hard clam, *Mercenaria mercenaria*, could be tagged using the antibiotic tetracycline, which fluoresces under ultraviolet light. However, they found that the tetracycline tag was ambiguous in *Mytilus edulis*. Larval and post-larval bivalves can also be marked with the vital stain alizarin sodium monosulfonate (Hidu and Hanks, 1968). The growing bivalve incorporates this stain into newly produced shell,
resulting in an easily seen and persistent band of reddish shell. Another tagging approach is to manipulate the algal diet, which is known to influence shell color in several gastropods such as *Haliotis* spp. and *Turbo* spp. (reviewed by Olsen, 1968). For instance, in *H. rufescens*, red algae cause the secretion of a red shell while brown and green algae produce white or light green or blue shell. Tegner (1987) found that because of this effect of diet on shell color, hatchery-produced abalone seed reared on a diet of diatoms or brown algae could be distinguished from wild seed, which is always reddish in color.

Genotypic shell polymorphisms have also been employed as biological tags. Chanley (1961) investigated the inheritance of the distinctive shell markings (reddish-brown zigzag lines on both valves) exhibited by the *notata* subspecies of *Mercenaria mercenaria*. Field plantings of this subspecies can be distinguished from naturally recruited individuals, which usually lack these markings (R. Kraus, pers. comm.). Unlike the other tags which eventually erode away, the genetic tag is continually produced as the individual grows.

Seed (1969) grew *Mytilus edulis* to a mean length of 17.5 mm in darkness and in light and found that the shell secreted in darkness was yellowish brown in color, while that secreted in the light was blacker. In our attempts to find a tagging method for *M. edulis*, we further investigated
the effects of light on pigmentation in post-larval mussels and its potential for creating recognizable bands.

MATERIALS AND METHODS

Experiment 1 (Growth Rates)

This experiment compared the shell pigmentation and growth rates of post-larvae reared under bright light of 11,000-16,000 lux versus semi-darkness of 1-40 lux. The post-larvae used had a mean initial length of 0.9 mm. Length was measured as the distance from the larval umbo to the posterior shell extremity. Each of the 55 post-larvae was reared in an individual 7 ml dish (1.5 x 3.0 cm, h x d). These 55 dishes were fully submerged in 3 cm of seawater in a 16-l tank (30 x 183 cm).

A fluorescent light fixture (1.5 m long with dual 40 w bulbs) was placed 2 cm above the water surface. One end (the dark end) was lined and covered with opaque black plastic. Twenty five dishes were arranged at this dark end of the tank and 30 at the opposite end, which was brightly lit. The 55 dishes took up 10% of the bottom surface area of the tank and were 3-5 cm apart. Water was pumped out of this tank at 4.5 l/min, through a cooling water bath, and back into the tank. Repeated measurements of algal density and temperature verified that this recirculating system prevented any significant differential heating or algal blooming in the 2 ends of the tank. Thus any confounding variability in environmental conditions at the different
ends of the tank were minimized. The water was changed every 3-5 days.

The concentration of the algal food used, Isochrysis galbana-Tahitian Strain (T-ISO), averaged 100 cells/μl. The temperature ranged from 17.3 to 23.5°C and averaged 19.7°C. After 17 days the lengths of all post-larvae were measured to the nearest 20 μm using a dissecting microscope at 50x, and an ocular micrometer. On day 17 the light was turned off and all post-larvae were reared in the dark for another 10 days. Final lengths were then measured.

Movement of the post-larvae out of their dishes was inhibited if the post-larvae were initially allowed to attach their byssal threads to their dish for 1 day before immersing the dishes in the water bath. The day before the experiment began, therefore, the post-larvae were added to their separate dishes (containing 6 ml of water), and allowed to attach. During the experiment, daily observations were made of the positions of the post-larvae. None of the dark treatment post-larvae left their dishes. However, there was some movement in the light treatment, with 12 of the 30 post-larvae leaving their dishes. These were excluded from the experiment.

Experiment 2 (Survival)

This experiment compared the survival of tagged versus untagged post-larvae in the laboratory using a larger sample size (n = 388) than used in Experiment 1. As in Experiment
1, all post-larvae (initial mean length = 0.5 mm) were reared in the same tank with one group being exposed to bright fluorescent light, the other to semi-darkness, using the same methods described above. The light was left on for 4 days. This was the length of time typically used to tag post-larvae. After this period, the 2 treatment groups were reared under dim light in separate 1-l dishes for 26 more days, at which time survival and the percentage of tagged individuals was measured.

**Routine Tagging Procedure with Fluorescent Light**

After metamorphosis, the post-larvae were reared in darkness to a length of 0.5-1.0 mm. One week after metamorphosis, a sheet of white PVC plastic (28 x 100 cm) was positioned horizontally in a trough of seawater (30 x 180 cm) 1-3 cm below the water surface. Between 10,000 and 100,000 post-larvae were then sprinkled over the 2800 cm² of PVC sheeting. The next day, the sheet was removed from the water and those post-larvae which had crawled to the edges or underside were removed. The sheet was then returned to the tank. At this time, 2 fluorescent light fixtures (1.5 m long with dual 40 w bulbs), were positioned directly over the post-larvae, 2-4 cm above the water surface. Water was pumped out of the trough and through a cooling water bath and back into the trough. This kept the temperature between 17-21°C and also helped circulate water over the post-larvae. T-ISO was fed at approximately 125 cells/µl every 1-2 days. Incident light intensity was 11,000-16,000 lux.
The growing post-larvae were held under this light for 4-5 days followed by 4-10 days of darkness. Between March 1985 and October 1986, this tagging procedure, with slight variations, was used 20 times to tag post-larvae from 7 different batches of larvae. Each of these batches was derived from a different group of parents which were collected from both Northern (Bodega Harbor and Tomales Bay) and Southern California (oil platform offshore of Santa Barbara).

**Routine Tagging Procedure with Sunlight**

In February and March, 1988, several batches of post-larvae were tagged using natural sunlight instead of fluorescent light fixtures. Approximately $0.5 \times 10^6$ post-larvae (0.4-0.8 mm) were sprinkled into a 1.3 x 2.6 m white water table which I filled with water to a depth of 7 cm and located in a greenhouse at the Bodega Marine Laboratory. The post-larvae were allowed to attach to the water table for several minutes before a gentle recirculating flow of seawater from a sump pump was begun. I added algal food to the sump as needed and a make-up flow of cold seawater was adjusted to keep the water from exceeding 22°C. After 4-9 days and nights in this greenhouse environment, the post-larvae were moved into a dark environment for the usual 4-10 day dark period.

**Field Trial**

A group of approximately 19,000 light tagged post-larvae (mean length = 1.9 mm) were allowed to attach to
substrata (sections of woven canvas fire hose) at a mean density of 41/cm². The total initial number of post-larvae was estimated using random quadrat sampling. These substrata were then attached to the underside of a plywood sheet which was floated on the water surface in 3-7 m of water in Marconi Cove on Tomales Bay, California, for 75 days (Sept.-Dec. 1985). After this time, the total number of mussels present was individually counted by breaking up the clumps and sorting. A sample of 809 of these mussels was collected randomly and each sampled mussel was examined for the presence or absence of a tag. In addition, the maximum length was measured to the nearest 0.1 mm with vernier calipers. Tags were recognized by their characteristic appearance (unpigmented shell on either side of the band) and by their location (≈1.0 mm from the umbo).

**Pigmentation Patterns of Wild Mussels**

Between Aug. 1985 and Aug. 1986, 319 *M. edulis* were collected from buoys, docks, rafts, and long lines in Bodega Harbor and Tomales Bay, California. These mussels ranged in length from 1.0-6.0 cm, though the majority were small (1.0-3.0 cm). Only mussels without erosion of the prismatic shell layer were used. I examined the umbones of each of these mussels at 12x under a dissecting microscope and measured with an ocular micrometer (to the nearest 85 μm) the length in the direction of growth from the umbo of the larval shell (prodissoconch) to the beginning of any darkly
pigmented shell. The occurrence of any band of pigmentation within 1.0 mm from the larval umbo was recorded.

RESULTS

Experiment 1 (Growth Rates)

Experiment 1 examined the effect of fluorescent light on the shell pigmentation and growth rate of *Mytilus edulis* post-larvae. Figure 1 shows post-larvae from Experiment 1 that had been grown for 17 days under either semi-darkness (1-40 lux) (Figure 1A) or continuous bright light (11,000-16,000 lux) (Figure 1B). All individuals grown under bright light secreted darkly pigmented shell over the 17 days. The length of these post-larvae on day 0 of this experiment could be determined from the length of the unpigmented shell. In contrast, all mussels grown under darkness secreted clear or nearly clear shell during the experiment. Since neither temperature nor algal concentration varied significantly between the 2 groups, the pigmentation response is presumably attributable to light alone. Also seen in Figure 1 are terminal byssal attachment plaques with short byssal hairs projecting out from the shells of the post-larvae.

The growth data of these post-larvae from Experiment 1 are given in Table 1. On day 0, the light and dark groups were not significantly different in length, averaging 896 μm (Student t-test; t=0.4, d.f.=41, p>0.5). During the 17-day experimental period the dark group grew significantly faster
Figure 1. Post-larval *Mytilus edulis* reared for 17 days under (A) semi-darkness (1-40 lux) or (B) continuous, bright (11,000-16,000 lux) fluorescent light. Bars are 1.0 mm.
Table 1. Initial mean lengths and growth rates (± S.D.) of post-larval *Mytilus edulis* during an experimental period (days 0-17) of either high (11,000-16,000 lux) or low (1-40 lux) light intensity and a subsequent control period (days 18-27) of low light for both groups. Comparisons were made using Student t-test.

<table>
<thead>
<tr>
<th>Light Treatment Group</th>
<th>Initial Length (± S.D.) (μm)</th>
<th>Growth Rate (± S.D.) (μm/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>days 0-17</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>x</strong></td>
<td>n</td>
</tr>
<tr>
<td>High</td>
<td>906 ± 137 18</td>
<td>34 ± 10 18</td>
</tr>
<tr>
<td>Low</td>
<td>890 ± 122 25</td>
<td>41 ± 8 25</td>
</tr>
</tbody>
</table>

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than the light group (Student t-test; t=2.5, d.f.=41, p<0.02). The mean growth rate of the dark group (41 μm/day) was 20.5% faster than that of the light group (34 μm/day).

During the 10 days subsequent to the experimental period, both groups of mussels were held in the dark. During this period, both groups showed rapid, though more variable growth, averaging 117 μm/day. No significant difference in growth rates between the 2 groups was detected during this subsequent period (Student t-test; t=0.3, d.f.=34, p>0.5).

In contrast to the initial 17 days of the experiment, when dark-reared post-larvae produced transparent shell, new shell growth during the final 10 days in the dark did contain some lightly colored pigmentation. This began to occur as the post-larvae reached approximately 1.5-3.0 mm.

Experiment 2 (Survival)

This experiment compared survival between tagged and untagged mussels in the laboratory over a 30-day period. Percent survival in the 2 groups was compared using Yate's X² test (Langley, 1971). Survival was nearly the same in both groups (X²=0.5, p>0.1). Seventy-seven percent of the tagged mussels (n=234) survived, compared to 78% survival for the untagged mussels (n=154).

Routine Tagging Procedure with Fluorescent Light

Figure 2 depicts the routine light banding process. Before exposure to bright light, the 0.8 mm post-larvae had a transparent shell (Figure 2A). After 4 days in the light,
Figure 2. *Mytilus edulis* at 3 stages of the tagging process: (A) immediately before rearing under light, (B) after being reared for 4 days under continuous, bright (11,000-16,000 lux) fluorescent light, and (C) after a subsequent 5 days in the dark. All bars are 1.0 mm.
the shell margins, where new growth had occurred, were pigmented (Figure 2B). During the next 5 days in the dark, all new growth was once again unpigmented. This created a banded appearance (Figure 2C). Figure 3 shows larger mus-sels (7.0-10.0 mm in length) that had been retrieved after a 1-month period in Marconi Cove. Their bands are still easily seen. All of the 7 batches of post-larvae used in the tagging studies uniformly showed the light-dependent pigmentation response.

The first attempts to tag large numbers of cohorts of post-larvae usually resulted in only 80-90% tagged. Un-tagged individuals appeared to be those shaded under clumps of other post-larvae or those that did not grow significant-ly. It was found that the percent tagged could be increased to greater than 95% by more uniform spreading out of post-larvae directly under the light source, and by extending the light phase to 5 days.

**Routine Tagging with Natural Sunlight**

This method of tagging is easier and more effective than the fluorescent light method. Even though the post-larvae spent each night in darkness, the intense daytime sunlight (whether during overcast or clear skies) was enough to stimulate the production of exceptionally black, distinct bands. Very large numbers of post-larvae \((0.5 \times 10^6)\) could easily be spread out over a large surface area of white water table without the need of large banks of light fix-tures. The percent tagged was very high (99%).
Figure 3. Tagged *Mytilus edulis* (7.0-10.0 mm in length) which had been transplanted to Tomales Bay 30 days earlier at a size of 1.0 mm. Bar is 7.0 mm.
The pigment bands produced by both tagging methods actually consisted of a periostracal band of reddish-brown pigment overlaying a blue pigment band in the prismatic shell layer. Together, these pigments produced the typical black color of *M. edulis*. The bands persisted as long as the umbo shell resisted erosion. The longest field trial performed to date lasted 101 days. No significant erosion to the bands occurred during this period.

**Field Trial**

Figure 4 is a size frequency histogram of the final lengths of tagged mussels retrieved after 75 days in Marconi Cove. The distribution appears bimodal. The patch of mussels was composed of a sheet of large mussels, relatively uniform in length, with many small mussels occurring below them and in the interstices (pers. obs.). Table 2 gives the total number and percent tagged data for days 0 and 75, and also the percent tagged data for the small (<10.0 mm) mussels. The total number of mussels did not change significantly during the field trial. The percent tagged also stayed constant (G-test of independence; G=0.6, d.f.=1, p>0.1) at approximately 83% for both the small and large modal groups.

**Pigmentation Patterns of Wild Mussels**

Of the 319 wild *M. edulis* examined, 16 (5%) had bands at ≤1.0 mm from the larval umbo. Thus bands similar to those produced in the laboratory also occurred in nature, though they were rare. The degree of pigmentation of the
Figure 4. Size frequency histogram of a random sample of 809 *Mytilus edulis* taken on day 75 of the field trial.
Table 2. Numbers and percent tagged *Mytilus edulis* at the time of transplantation to Tomales Bay and 75 days later. The total number on day 0 was estimated with random quadrat sampling. The 95% confidence interval of this estimate is given. The day 75 total number was determined by direct counting of all individuals. A subset of the total (n) was measured for length and determination of the percent tagged. Also shown is the percent of tagged mussels that were less than 10.0 mm in length on day 75.

<table>
<thead>
<tr>
<th>Day</th>
<th>Total Number</th>
<th>Percent Tagged</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>18,739±736</td>
<td>83.8</td>
<td>702</td>
</tr>
<tr>
<td>75 (total)</td>
<td>19,207</td>
<td>82.2</td>
<td>809</td>
</tr>
<tr>
<td>75 (&lt;10.0 mm)</td>
<td>-</td>
<td>84.2</td>
<td>492</td>
</tr>
</tbody>
</table>
early shell of these mussels was found to vary between different habitats, as shown in Table 3. Mussels from surface long lines in Marconi Cove were more likely to be naturally banded at ≤1.0 mm from the umbo (G-test of independence; G=5.28, d.f.=1, p<0.025) and tended to be pigmented nearer to the umbo (Student t-test, variances not pooled; t=6.0, p<0.001) than did the raft population. The long line environment seemed less shaded than did the raft environment. When the mussels from all the different habitats sampled were pooled, the mean length (± S.D.) of unpigmented shell was 1.8 ± 1.5 mm.

DISCUSSION

Experiment 1 showed that, for post-larvae less than 1.5-3.0 mm in length, light is needed to induce the deposition of both the periostracal and prismatic pigments. This result was consistently seen in the 23 routine tagging trials of 8 different batches of post-larvae using both fluorescent and natural light. The production of shell pigmentation could be turned on and off by varying the light intensity. After a shell length of approximately 3 mm is reached, the role of light on pigmentation becomes less dramatic since some pigmentation occurs at this length in nearly complete darkness. Seed (1969) showed, however, that light continues to exert an effect on the intensity of pigmentation up to a length of at least 23 mm.
Table 3. Comparison of pigment patterns of wild *Mytilus edulis* from 2 habitats in the same cove. The mean distances (± S.D.) from the larval umbo to the first band of pigmentation in the prismatic shell layer are given as well as the number of mussels that had pigment bands <1.0 mm from the larval umbo.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Distance to First Pigmentation (± S.D.) (mm)</th>
<th>Number with Bands &lt;1.0 mm from Umbo</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long Lines</td>
<td>1.00 ± 1.04</td>
<td>8</td>
<td>88</td>
</tr>
<tr>
<td>Raft</td>
<td>2.92 ± 2.33</td>
<td>0</td>
<td>61</td>
</tr>
</tbody>
</table>
Genetics also plays an important role in the shell color of *Mytilus edulis*. The crossing experiments of Innes and Haley (1977) showed that genotypic, rather than environmental differences, were responsible for the production of a light brown morph of *M. edulis*. This brown morph, which is rare in Tomales Bay and Bodega Harbor, was not investigated in my study. Thus it is not yet known how light affects the expression of color in this morph. It seems likely, however, that light will affect the intensity of expression of the individual mussel's genetically determined pigments.

My observations suggest that the early shell of wild mussels provides a record of the light environment encountered by those individuals near the time of recruitment. From the comparison of mussels from the long line versus the raft (Table 3), it is clear that this early light environment is variable, reflecting the variability in microhabitats chosen by larvae or post-larvae. The under-raft habitat was probably more shaded than was the long line habitat. On the whole, however, the pooled data showed that wild *M. edulis* spent their early post-settlement days in shaded environments since, on average, the first 1.8 mm of shell was unpigmented. This is consistent with the work of Bayne (1964) which showed that crawling pediveligers were strongly negatively phototactic. The well-known preference of pediveligers and early post-larvae for filamentous or highly rugose substrata (Blok and Geelen, 1958) would also tend to concentrate these individuals in shady microhabitats.
The chemical structures of the pigments in mussel shells are still unclear. The reddish-brown periostracal pigment is probably a melanin, sclerotin, or possibly an ommochrome (Fox, 1983). Less is known about the blue prismatic pigment. For the post-larvae, these pigments may serve to block out UV irradiation which is well known to cause mutations and tissue damage (reviewed by Porter, 1967). Mitton (1977) investigated shell color in M. edulis and its adaptive significance. He found that of 2 color morphs of mussels which co-occur on the eastern seaboard of the United States, the less pigmented morph was more abundant in warmer latitudes, whereas in colder environments the darker morph predominated. In addition, under controlled conditions, the dark shelled morph absorbed significantly more heat and attained higher internal temperatures than did the less pigmented phenotype. The differential occurrence of these 2 genetic color morphs could thus be explained by their differential rates of heat absorption. In Virginia, for instance, where death due to high summer temperatures is common, the less pigmented morph would have an advantage.

In contrast to the conclusions of Mitton's study, the present study showed that shell pigmentation of post-larvae was greatest when light intensity was greatest. Thus Mitton's heat protection hypothesis does not seem to apply to this post-larval pigmentation phenomenon which is better explained by an inducible UV light protection hypothesis.
In the absence of bright light, the shell pigments are not needed at all and the post-larva may redirect the energy and materials needed to produce these pigments into other physiological functions, such as growth.

Experiment 1 showed that post-larval growth in the light was slower than in the dark. It is well known that light has an inhibitory effect on growth in *M. edulis*. Though an earlier study (Dodd, 1969) gave contradictory results, Nielsen and Stromgren (1985) showed clearly that as long as food is not severely limiting, growth is enhanced by darkness. They found that growth in darkness was 20% greater than that in bright natural sunlight. This figure agrees well with mine of 20.5% for continuous fluorescent light. Thus the present study on small post-larvae extends the earlier work which focused on larger mussels. Light probably reduces growth rate by inhibiting ingestion rate (Nielsen and Stromgren, 1985). For the post-larvae, growth may be further inhibited by light if the darkly pigmented shell produced under bright light is energetically more costly than clear shell.

The tagging procedure described in this study can thus be expected to inhibit growth rate slightly. However since this effect was shown to be eliminated upon removal of the post-larvae from light (Table 1), the tagging procedure should not have any permanent effect on growth. Likewise, no effect of the tag on subsequent survival was found in either the laboratory or the field (Experiment 2; Table 2).
Finally, the tag is persistent enough for field studies of durations on the order of months. After several years, however, the umbones of mussels can become eroded, especially in exposed environments (pers. obs.).

A potential complication of the light banding procedure is that "tagged" individuals do occur occasionally in nature, particularly in environments having high light intensities. The highest occurrence found here was 9% in the surface long line habitat. In most of these wild "tagged" mussels, the band occurred randomly from 0.3-1.0 mm from the umbo and was usually closely followed by more bands of dark pigmentation. Thus, by producing a pigment band at a narrowly defined position (e.g. 0.6-0.8 mm) and by producing a relatively broad band of unpigmented shell after the pigment band, these hatchery-produced tags will be easier to distinguish from wild "tags". For most purposes however, wild "tags" seem to be rare enough not to cause any significant errors.

Another characteristic feature of post-larval mussel shells is the terminal byssal attachment plaques and hairs shown in Figure 1. Board (1983) suggested that these hairs developed when aggregated post-larvae attached (and subsequently detached) to each other's shells. Since the post-larvae in Experiment 1 were reared in isolation from one another, each individual must have produced its own set of these projecting byssal hairs. Ockelmann (1983) verified
that for *Mytilus* sp. and *Modiolus* sp. at least, these projections were in fact byssal hairs, secreted by the foot, rather than the mantle. Hair-like projections occur on many other mytilids and are often very pronounced. Bottjer and Carter (1980) showed that in *Modiolus rectus* these hairs have a sensory function. They can also serve to repel predators, such as drilling gastropods, as was shown for *Modiolus modiolus* (Wright and Francis, 1984).

The field trial (Figure 4; Table 2) gave an example of how the tag can be used to help interpret data. Since percent tagged did not change significantly during the course of the experiment, the bimodal peak could not be attributed to new recruitment. The density of mussels in this patch was high (41/cm²) with larger, fast growing mussels forming a mat, the interstices of which were occupied by the smaller, slower growing mussels. Apparently initial differences in growth rates were further accentuated by the fast-growing mussels' ability to confine the slower growing ones to the interstices that have less water circulation.

The optimal wavelength and intensity of light for producing the best tag needs more study. Work to date does suggest that short UV light is not necessary for the pigmentation response since the tag was elicited by light from fluorescent fixtures which were encased in clear plastic covers. These covers screen out any UV light produced. However, natural sunlight produced a darker, more distinct
band. This result is probably due to the high intensity of
natural light (often greater than 100,000 lux) relative to
that of the fluorescent light fixtures used (16,000 lux).
The tagging method described in this study was shown to be
practical for mussel post-larvae. Its usefulness with other
species remains to be tested.

For the ecology of most marine invertebrates, the early
post-larval stage is poorly understood. For instance,
Spight (1972), working with Nucella lamellosa, quantified
larval abundance (in egg cases) and yearling abundances, and
from these data estimated the first year's mortality.
Actual direct counts and manipulations of the small, cryptic
individuals, however, was not possible. The approach of
producing, tagging, and then "seeding" early post-larvae in
the natural environment may help in better understanding
this inconspicuous life stage.

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III. FISH PREDATION AND SEASONAL PATTERNS
OF EARLY MORTALITY

INTRODUCTION

Though others have observed fish predation on mussels, the impact and characteristics of this interaction have not received much attention (see Suchanek, 1986 for a review of mussel predators). Okamura (1986) observed mixed schools of surfperch (notably *Ephiotoca lateralis* and *Damalichthys vacca*) in San Francisco Bay feeding on small, naturally settled *Mytilus edulis* (<20 mm) among the experimental settlement plates which at that time (early July) had a 100% cover of mussels. By mid-July, mussel percent cover had dropped to 15%. There have also been more casual observations of fish predation on farmed mussels in Puget Sound (P. Jeffers, pers. comm.), Southern California (D. Glenn, pers. comm.) and India (Appukuttan, 1980). Despite these observations, it is still uncertain how important fish predation is to mussel populations relative to other mortality factors.

In attempting to use small (1 mm), hatchery-reared mussel spat to start crops in Tomales Bay, I often noticed that many spat disappeared soon after planting. Myers (1980), Nie *et al.* (1979), and J. Richards (pers. comm.) also observed similar losses of planted hatchery reared mussel spat. I observed shiner surfperch, *Cymatogaster*
*aggregata*, feeding on one of the planted crops in Tomales Bay during the summer of 1985 and became interested in the effect that these fish predators exerted on these crops, relative to other factors.

For instance, small post-larval mussels, such as those used in the present study, are known to be capable of secondary planktonic migrations, using a "drifting byssus" which reduces sinking velocity (Bayne, 1964; Sigurdsson et al., 1976; Blok and Tan-Maas, 1977; see Lane et al., 1985 for review). The spat used in the present study were observed in the laboratory exhibiting this behavior. Lane et al. (1985) suggest that a significant part of the post-larva's life up to a size of 2 mm might be spent undergoing one or repeated migrations. However, it still is not known how prevalent this behavior is in the field or how important it is in explaining the disappearances of spat. For instance, Seed (1969a) also found evidence for post-larval planktonic migration in the field. He attempted to quantify disappearance rates of "very small mussels", but was unable to tag them, due to their small size (Seed, 1969b).

The purpose of this study was to determine the importance of fish predation relative to other possible factors affecting apparent mortality, such as post-larval migration. Specifically, I investigated the identity of the predators, their contribution to the observed losses, their seasonality, and their size selectivity.
MATERIALS AND METHODS

Three different approaches were used:

1. Field caging experiments at 2 sites and over 2 years.

2. Fish trapping, gut content analysis, and visual observations of predation in the field.

3. Laboratory feeding trials.

1. Field Caging Experiments

The aim of these experiments was to measure the losses of mussels from tiles that were originally seeded with known numbers of tagged spat and then placed in the sea under various caging treatments and times of year. These were short term experiments, usually lasting for one month.

The spat were produced in the hatchery and light-tagged as described in Chapters I and II. They were maintained in the laboratory in tanks of flowing seawater, either attached to baffles or to mesh-bottomed cylinders, until needed for an experiment. Isochrysis galbana (T-ISO) was fed every 1-3 days to keep the spat growing very slowly (0.1 mm/month). Before each experiment, a sample of 10-100 spat was taken for length measurement, using an ocular micrometer on a dissecting microscope. The initial mean length for all of the experiments averaged together was 1.1 mm (range=0.4-2.4 mm).

Before each experiment, a measurement was made of the percent of the spat that were tagged. A sample of 100-500
spat was taken for this purpose and each spat was examined under the dissecting microscope for the presence of a distinct band. The mean initial percent tagged for the 16 experiments was 89.9% (range=44%-98.9%).

The spat were sprinkled onto red clay tiles (7.6 x 7.6 cm, American Olean Quarry, USA) that were in a water table under 5 cm of water. These tiles had a grid penned on their surface to facilitate counting. After letting the spat attach for several minutes to an hour, flow-through sea water was turned on, and the water depth was increased to about 25 cm and was agitated continuously using aeration for 2-12 days in order to stimulate firm byssal attachment. Figure 1 shows a portion of a seeded tile.

The day before initiation of an experiment, the spat on the tiles were counted under a dissecting microscope at 12x or 25x by moving the field of vision down the grid lanes on the tile. The mean initial number of spat per tile was 520 (range=178-1278, n=174 tiles). On the first day of an experiment, the tiles were wired (3 cm apart) onto plastic racks made from light fixture grating (see Figure 6).

One of 4 caging treatments was randomly assigned to each tile. These treatments were uncaged, cage-control, large mesh cage, and small mesh cage. There were 3-4 replicates per treatment. The cages consisted of a heavy (2 mm diameter) stainless steel wire frame, 10.5 cm on a side, and 4 cm tall, to which was sewn netting. The large
Figure 1. Close up photograph of a seeded tile ready for a field experiment. These square clay tiles measured 7.5 cm on a side. The parallel lines, which were used to facilitate counting of the spat, were spaced 6 mm apart. The tagged 1 mm spat were bysally attached over the tile surface.
mesh netting was multi-purpose garden netting (Ross Co.) which is made of thin (0.4 mm) diameter black plastic fibers that form square mesh holes, 22 mm on a side. The small mesh netting was made from this same material, but each mesh hole was divided in half by a sewn-in string so that the mesh holes were rectangular, 22 mm by 11 mm. The cages were fixed in place over the seeded side of the tile. The cage-control treatment consisted of the wire cage frame without any netting.

The racks and seeded tiles were driven to Marconi Cove, and were kept damp and shaded en route (45 minutes). They were then suspended, with the tiles hanging vertically from either the dock or the raft, 0.3-1 m under the water surface. The racks were 1 m and 3.3 m above the bottom at mean lower low water at the dock and raft respectively. The racks were positioned away from floats and ropes so that crabs could not crawl onto the racks.

Depending on the experiment, the racks were left in the Cove for 8-43 days. After this time, the tiles were returned to the laboratory and destructively sampled, as this was the only way of accurately counting the mussels. Most of the macroscopic organisms were scraped off the tiles and the mussels were sorted from the other sessile species (mainly bryozoans, hydroids, ascidians and tubicolous amphipods). The mussels were collected and washed onto a 150 μm screen with a grid penned on it. All the
mussels were counted under a dissecting microscope at 6x or 12x magnification. The first 30 mussels on the screen were examined for tags for each tile. The shell lengths of the first 7-10 mussels from each tile were measured using the ocular micrometer.

The initial and final percent tagged data were compared using the G-test of independence (Sokal and Rohlf, 1969). If a significant (p<0.05) reduction in percent tagged occurred, then the final number of surviving hatchery reared mussels (N_s) was calculated by multiplying the final number of mussels by an adjustment factor. This factor was the ratio of the final percent tagged over the initial percent tagged:

\[ N_s = N_t \times \frac{\%T_f}{\%T_i} \]

Where N_s is the number of survivors,
N_t is the total final number of mussels,
\%T_f is the final percent tagged, and
\%T_i is the initial percent tagged.

The mortality occurring on each tile was calculated as the ratio of the final number of survivors over the initial number. These mortality data were transformed by the arc sine transformation (Sokal and Rohlf, 1969) in order to increase their normality. Parametric ANOVA was performed and when the variances were found to be heterogeneous (by examining residuals and by Bartlett's test), or the data were not normally distributed (as determined by normal probability plots), then the Kruskal-Wallis nonparametric
ANOVA was performed. A posteriori pairwise comparisons were made using Fisher's Protected Least Significant Difference test (Geng and Hills, 1980). For graphical presentation of the mortality data, the transformed treatment means and 95% confidence limits were back-transformed onto the original scale of measurement.

The mean growth rate of spat during each experiment was estimated from initial and final length measurements, by calculating the slope of the linear regression of length vs day.

Sixteen of these clay tile caging experiments were performed. In addition, I conducted one large scale caging experiment using untagged spat attached to scour pad material. This material was often used when starting large scale crops as described in Chapter V. Table 1 summarizes where and when the 17 caging experiments were conducted. For clarity of presentation, the specific details of the methods used in these experiments are given below in the appropriate results sections.

2. Fish Trapping and Observations

Between September 1986 and September 1987, the relative abundances of several species of surfperch (Embiotocidae) were measured on a roughly weekly basis at the raft site using 4 replicate fish traps. These traps were of the minnow trap design, but larger, and were made of 13 mm mesh black polyethylene "Duronet" screening (Nalle Plastics,
Table 1. Summary of the 17 field caging experiments conducted in Marconi Cove.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Location</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2</td>
<td>Dock</td>
<td>Aug.–Oct. 1985</td>
</tr>
<tr>
<td>3 (Depth)</td>
<td>Raft</td>
<td>May 1986</td>
</tr>
<tr>
<td>4-8</td>
<td>Raft</td>
<td>Apr.–Sept. 1986</td>
</tr>
<tr>
<td>17 (Lg. Scale)</td>
<td>Raft</td>
<td>Aug. 1987</td>
</tr>
</tbody>
</table>
Inc., Austin, TX). They were cylindrical, 122 cm long and 30 cm in diameter, with 2 inward pointing cones at each end. The inner openings into the traps were 9 cm in diameter. The traps were baited with approximately 5 medium (6-8 cm) *Mytilus edulis*, cut in half, and were suspended 1 m below the surface at the dock or raft for 1 hour. The captured fish were identified and counted. All the shiner surfperch, *Cymatogaster aggregata*, from one or more of the traps, depending on the size of the catch, were measured for total length.

I also examined the gut contents of captured surfperch on 8 different occasions between 20 April 1987 and 13 August 1988. Between 3 and 23 surfperch guts were examined on each of these 8 sampling dates. The gut contents were analyzed by counting the number of mussels in the gut, estimating their lengths under a dissecting microscope with an ocular micrometer, and noting the dominant food item and its approximate percent contribution to the total contents.

Between 1985-1988, field observations were made, usually for several hours each week, and especially upon immersion of a new experiment or crop. At these times, surfperch were observed for feeding behavior on the planted spat, and the spat were observed in order to determine if any fell off upon immersion. These observations were made from the surface while lying on the raft or dock.
3. Laboratory Feeding Trials

In addition to the field work, prey size selection by shiner surfperch, *Cymatogaster aggregata*, feeding on *Mytilus edulis*, was examined in laboratory aquaria. The surfperch were divided into 2 size classes: medium (9-11 cm total length) and large (12-14 cm). The small, young-of-the-year were not studied. Fifteen medium fish were placed in one flow through 150 l aquarium and 14 large fish in a 200 l aquarium. These fish were maintained for several months and were fed brine shrimp and mussel meat while the study was conducted.

The feeding trials were carried out by immersing a rack of tiles seeded with different size classes of mussels into the aquaria and measuring percent eaten of each size class after 30 minutes or 30 hours. Consumed mussels were not replaced during a run so that I could determine not only what sizes the shiner surfperch preferred, but what sizes they could eat once the preferred sizes were all gone. In each run, 8-10 tiles were first seeded with 39-50 mussels each. Each tile received a different size class of mussel. Thus each size class was represented by just one unreplicated tile. The size classes varied between 1 and 10 mm in length, at 1 mm intervals. The mussels for each size class were selected by measuring each one under a dissecting microscope with an ocular micrometer and using only those mussels that fell within 0.3 mm of a size class. The fish were not fed for 24 hours prior to a run. The
seeded tiles were randomly arranged on a rack which was then immersed in the aquarium. Feeding behavior was observed during the first several hours. After 30 hours, the aquarium was cleaned out to retrieve fallen mussels and shell fragments.

Six of these feeding trials were conducted, 3 on the medium fish and 3 on the large fish. Two of the medium fish trials were sampled at 30 minutes, as well as the usual 30 hours. Sampling was performed simply by removing the rack of tiles and non-destructively counting all the mussels on each tile. Non-destructive sampling was possible in this case because there was a maximum of only 50 mussels per tile.

RESULTS

1. Field Caging Experiments

   A. Summary of Tagging Data.

   Figure 2 summarizes how the percent tagged changed during each of the 16 tile caging experiments. Points significantly above the zero line indicate recruitment of untagged, wild spat, while points significantly below the line indicate an increase in the percent tagged which could be caused by selective mortality of untagged spat.

   Experiments 7 and 15 showed a significant (p<0.05) reduction in percent tagged, indicating recruitment by wild spat. In Experiment 7, conducted in July-August, 1986, percent tagged decreased 12 percentage points.
Figure 2. The change in percent tagged during each of the 16 tile caging experiments. The y-axis is the initial percent tagged ($%T_i$) minus the final percent tagged ($%T_f$). The 3 arrows mark those experiments where significant changes in percent tagged occurred. A significant deviation above the zero line indicates recruitment onto the tiles by wild *Mytilus* sp. Positive identification to species of these small wild spat was not possible.
Figure 2
Consequently, the final number of mussels was adjusted as described in the methods section. The adjustment factor equaled 0.87, thus 87% of the final mussel count was considered to be hatchery reared. Similarly, the adjustment factor for Experiment 15, conducted in June, 1987, was 0.94, suggesting that a small amount of wild settlement occurred.

In contrast, Experiment 10 showed a significant (p<0.05) increase in percent tagged. At the onset of this experiment, it was noted that the untagged spat were smaller and less viable looking than the tagged spat. These may have died disproportionately during the experiment, driving the percent tagged up. Thus I did not adjust the final number of mussels since no natural settlement occurred. The other 13 experiments showed no significant changes in percent tagged.


Experiment 1 was conducted at the dock site between August 16-26, 1985. Table 2 shows the raw data from this experiment. A significant cage effect was found (ANOVA; F=132, d.f.=2,6, p<0.001). The uncaged (open) and cage-control (frame) tiles had uniformly high (94-97%) mortality rates, and these 2 treatments did not differ significantly from one another (Fisher's Protected Least Significant Difference (PLSD); p>0.05). In contrast, mortality on the caged tiles was low (0-10%), and differed significantly from the other 2 treatments (PLSD; p<0.001). This caging
Table 2. Initial and final numbers of spat, and percent mortality, on the 9 tiles of Experiment 1, conducted between August 16-26, 1985 at the dock.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial Number</th>
<th>Final Number</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open</td>
<td>774</td>
<td>34</td>
<td>95.6</td>
</tr>
<tr>
<td></td>
<td>761</td>
<td>47</td>
<td>93.8</td>
</tr>
<tr>
<td></td>
<td>507</td>
<td>14</td>
<td>97.2</td>
</tr>
<tr>
<td>Frame</td>
<td>714</td>
<td>19</td>
<td>97.3</td>
</tr>
<tr>
<td></td>
<td>666</td>
<td>34</td>
<td>94.9</td>
</tr>
<tr>
<td></td>
<td>868</td>
<td>40</td>
<td>95.4</td>
</tr>
<tr>
<td>Cage</td>
<td>599</td>
<td>618</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>447</td>
<td>404</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td>590</td>
<td>632</td>
<td>0</td>
</tr>
</tbody>
</table>
effect corroborated my visual observations made earlier that month of *Cymatogaster aggregata* picking off nearly all the mussels on coir rope and tiles suspended from the dock. On 2 of these caged tiles, there were more spat counted on day 10 than on day 0. This was due to counting error. The day 0 counts underestimated the actual number present because they were done without disturbing the clumps of spat which hid some underlying individuals. In subsequent experiments, this problem was minimized by removing and discarding the denser clumps of spat before counting.

Experiment 2 was conducted between August 26 and October 8, 1985 (43 days) at the dock to see if the cage effect would be repeated. This experiment had eight replicate tiles for each treatment. It was sampled twice, on days 10 and 43, by removing 4 randomly chosen tiles from each treatment. In this experiment, the spat were seeded onto the ridged side of the tiles rather than the smooth side, as in Experiment 1. This was done in order to investigate how a rough texture might affect the spat mortality. The ridges were 2 mm tall and spaced 2 mm apart.

The results were very similar to those of Experiment 1, heavy losses occurring rapidly on the unprotected tiles (Figure 3). There were significant caging effects both on day 10 (ANOVA; $F=172$, d.f.$=2,9$, $p<0.001$), and on day 43 (ANOVA; $F=75$, d.f.$=2,9$, $p<0.001$). The open and frame treatments did not significantly differ from each other on either day (PLSD, $p>0.05$). The cage treatment, on the
Figure 3. Experiment 2. Mean percent mortality of planted spat (± the 95% confidence interval) from the 3 caging treatments over a 43 day period at the dock using ridged tiles. "Open" denotes the uncaged treatment, and "frame" denotes the cage-control treatment. These 2 designations also apply to the other caging experiments described later. "Cage" here denotes the large mesh cage.
other hand, had significantly lower mortality than the other 2 treatments on both days (PLSD; p<0.001). It was noticed that the spat often crawled up and out of the grooves between the tile ridges, making them vulnerable.

C. Experiment 3: The Effect of Depth.

This experiment was conducted in May of the following year (1986) at the newly constructed raft. It compared losses of spat from uncaged tiles held at 3 different depths over an 8 day period. There were 3 replicate tiles per depth. These depths were 0.3, 0.8, and 1.5 m from the surface. The results are shown in Figure 4. Water depth had a significant effect on losses of spat (ANOVA; F=115, d.f.=2,6, p<0.001). At 0.3 m, losses averaged 57%, while at 0.8 m and 1.5 m, losses approached 100%. Losses at the shallowest level were significantly less (PLSD; p<0.001) than they were at the 2 deeper levels, which did not differ significantly from each other (PLSD; p>0.05).

Very possibly, if this experiment had been left in the water for several more days or weeks, the mortality of the shallowest spat would have risen to the high levels observed for the deeper spat. But this 8 day experiment showed that the rate of mortality was less nearer the surface. This result agreed qualitatively with my observations of schools of surfperch, usually Cymatogaster aggregata, in Marconi Cove. These surfperch usually stayed out of the upper 0.5 m of water.
Figure 4. Experiment 3. Mean percent mortality of planted spat (± the 95% confidence interval) on uncaged (open) tiles suspended at 3 depths for 8 days (May 22-30, 1986) under the raft.
D. Experiments 4-8: Summer of 1986.

These caging experiments were conducted at the raft between April and September, 1986. Each experiment lasted for 30 to 33 days. Thus the duration of these experiments varied slightly, one from another. This variation had to be taken into account in order to compare the different experiments. This correction was made by standardizing the mortality data to a 31 day period. For instance, if a tile had 50% mortality over a 33 day period, this was estimated to be equal to 47% mortality (50*31/33) over a 31 day period. As with Experiments 1 and 2 described above, these experiments also compared losses of spat from uncaged tiles, cage control tiles and caged tiles. In Experiment 8, the small mesh cage was used for the first time, in addition to the large mesh cage.

The results of this group of experiments are shown in Figure 5. In late April to early May (Experiment 4) there was no cage effect (ANOVA; F=0.1, d.f.=2,6, p=0.94), but by late May to early June (Experiment 5) there was a significant cage effect (ANOVA; F=17.1, d.f.=2,6, p=0.003). In this experiment, the uncaged tiles had significantly higher (PLSD; p<0.05) losses of spat than did the cage control and cage treatments, which did not differ significantly (PLSD; p>0.05). In early July (Experiment 6), the uncaged tiles had uniformly heavy losses, and so an extreme caging effect was produced, similar to the previous summer's results at the dock. This experiment is shown on the final day in
Figure 5. Experiments 4-8. The mean percent mortality (± the 95% confidence interval) in 5 separate 1 month-long caging experiments conducted at the raft between April and September, 1986. Each group of points represents the results of an independent experiment. Open circles = open treatment; filled circles = frame treatment; filled triangles = large mesh cage treatment; open triangle = small mesh cage treatment.
Figure 6. The black colored mussels are visible on tiles 1, 3, and 6 counting up from the bottom. These are the cage treatment tiles.

The early August experiment (Experiment 7) also showed a significant caging effect (ANOVA; $F=7.7$, $d.f.=2,6$, $p=0.02$). The cage treatment had significantly (PLSD; $p<0.05$) lower losses of spat than did the other 2 treatments, which did not differ from each other significantly (PLSD; $p>0.05$). However, the magnitude of this cage effect was small in this experiment; the cage treatment had only a slightly lower loss than the other 2 treatments. For some reason, the losses under the cage tiles went up relative to previous experiments. During this experiment, many small, young-of-the-year surfperch were seen around the farm.

The small mesh cage was used for the first time in Experiment 8. This experiment compared mortality under the frame (cage control), the large mesh cage, and the small mesh cage. The open (uncaged) treatment was not used. A significant caging effect was found (ANOVA; $F=168$, $d.f.=2,6$, $p<0.001$). Like the previous experiment, the frame and large mesh cages both had high mortality. These 2 treatments did differ from each other, however; the mortality under the frames was slightly greater than under the large mesh cages (PLSD; $p<0.05$). The small mesh cages, on the other hand, had much lower mortality than either of the other treatments (PLSD; $p<0.001$).
Figure 6. Experiment 6 on day 32, immediately after removal from the water. This rack had been suspended 0.6 m under the raft, in an orientation such that all tiles were at the same depth. The rack had a random arrangement of 3 replicates of each of the 3 caging treatments. This rack design was used for Experiments 1-8. The caged treatment tiles were numbers 1, 3, and 6 counting up from the bottom. The black masses on these 3 tiles were clumps of mussels.
E. Experiments 9-16: Raft Site.

This group of experiments was conducted at the raft between October 1986 and September 1987, using all 4 caging treatments. Figure 7 shows the results of these experiments. Between October and April (Experiments 9-13), mortality varied greatly from month to month, but ANOVA's showed no significant caging effects within any experiment. The May-June experiment (Experiment 14) however, showed a significant cage effect (Kruskal-Wallis test statistic=9.1, d.f.=3, p=0.03). The open and framed tiles showed uniformly high (99-100%) mortality. In contrast, the large and small mesh cages had mean mortality rates of only 51-53%. These 2 types of cages (large and small mesh) protected the mussels equally well. The appearances of the tiles in this experiment on the final day are shown in Figure 8. Clusters of mussels are visible only on the caged tiles. Counting up from the bottom, these are tiles 2 and 3 on the left hand rack, tiles 3 and 4 on the middle rack, and tiles 1 and 2 on the right hand rack.

The subsequent experiment (Experiment 15) in June-July also showed a significant caging effect (Kruskal-Wallis test statistic=8.6, d.f.=3, p=0.035). Here all but the small mesh treatment suffered 100% losses. When this experiment was immersed at the raft in June, 1987, a school (about 50-75) of small surfperch, probably Cymatogaster aggregata, was observed quickly picking off the spat on the open and frame tiles during the first hour after immersion.
Figure 7. Experiments 9-16. A. Mean percent mortality (± the 95% confidence interval) on open tiles (open circles) and frame tiles (solid circles) for 8 different one month-long caging experiments performed between October 1986 and September 1987 at the raft. B. Mean percent mortality (± the 95% confidence interval) on large mesh cage tiles (solid triangles) and small mesh cage tiles (open triangles) for the same 8 experiments. The numbers (9-16), which appear next to each pair of points, designate the experiment.
Figure 7
Figure 8. Experiment 14 on day 31, immediately after removal from the water. Each of these 3 racks of 4 tiles each had been suspended at a depth of 0.6 m, at different locations under the raft. Each rack contained one replicate of each of the 4 caging treatments, randomly arranged. This rack design was used in Experiments 9-16.
All these tiles were completely denuded of spat during this time, whereas both cage treatment tiles appeared untouched. I returned after another hour, and observed that all the large mesh cage tiles were devoid of spat as well, but the small mesh cage tiles still appeared unchanged.

The August experiment (Experiment 16) shown on the right in Figure 7 also had a strong caging effect (Kruskal-Wallis test statistic=9.7, d.f.=3, p=0.02), with the large and small mesh cages both having low losses relative to the uncaged treatments which once again had uniformly high losses. Thus the large mesh cage was effective in this experiment.

F. Growth Rates of Spat.

Spat growth rate was measured in Experiments 4-16 in order to determine how growth of small spat changed seasonally. Figure 9 summarizes these growth rates. This figure shows the seasonal fluctuation in growth rate and its correlation with sea surface temperature in Marconi Cove. Each growth rate point is the mean growth rate (± 95% confidence intervals) that occurred during a tile caging experiment at the raft. In both years, growth rate peaked in late May and early June to a mean of 5.4 mm/month. Growth reached a minimum of 0.2 mm/month in late December and early January. The three different symbol types represent the 3 different batches of spat used. For instance, the solid triangles are for Experiments 11-16, which all used spat from the same spawn.
Figure 9. The upper curve shows the sea surface temperature (°C) at Marconi Cove between April 1986 and September 1987. The lower points are the growth rates of spat in Experiments 4-16 at the raft, Experiment 4 being the left-most solid circle and Experiment 16 being the right-most solid triangle. The 3 symbol types represent the 3 different larval batches used. Each of these points is aligned on the x-axis at the midpoint of the experimental period.
Figure 10 shows in greater detail the growth of this batch of spat as it was used for Experiments 11-16. Fertilization occurred on October 7 and settlement (arrow) occurred 18 days later. During November, the spat were tagged and in the process grew up to nearly 1 mm. From then on they grew very slowly in the laboratory. Periodically, portions of this batch were transplanted to the field (for Experiments 11-16) where they grew at varying rates. During the spring and summer, they grew rapidly as soon as they were moved to the field, though they had been held in the laboratory for up to 10 months.

G. Large Scale Caging Experiment.

In addition to the tile experiments described above, the effect of fish predation on a crop of 300,000 spat was examined. Chapter V describes the production of some of these larger scale crops. One material that was used frequently was medium duty green scour pad (Loren Products, Lawrence, MA). This fibrous material provided crevices for the spat to occupy.

This experiment was conducted as follows. A clean, homogeneous mass of approximately 300,000 small (0.5 mm), untagged spat was divided into 10 equal portions by wet weight, using a top loading Mettler balance. Each portion was then sprinkled onto a piece of scour pad, 20 x 100 cm, immersed in a tank of flowing sea water. The seeded pads were left in the tank for 3 days to allow time for the spat
Figure 10. Growth of the batch of spat used for Experiments 11-16. The arrow marks the peak day of metamorphosis. The y-axis is the mean length (+95% confidence interval). Each branch point marks when a portion of the batch was taken and used in a field experiment.
to attach firmly. Five of these pads (haphazardly selected) were then slipped inside individual lengths of black polyethylene "Duronet" heavy weight shellfish and bait bag net tubing (Nalle Plastics Inc., Austin, TX) which has a 14.3 mm (9/16 in) mesh diameter. The net tubing bent the pad strips into a U-shape, with the spat on the inner, concave surface, away from the netting.

The 10 pads were suspended from the raft on August 14, 1987 in the upper 2 m of water, 30 cm apart. The 2 treatments were alternated along the row. On days 4 and 14, a 15 cm piece was cut off the bottom of each pad. These were brought back to the laboratory and subsampled. Subsampling was performed by cutting out 4 randomly selected pieces (quadrats), 3 x 10 cm each. Each quadrat was torn apart and shaken in a 20% bleach solution for 4-6 minutes. The bleach helped to sever byssal attachments (Davies, 1974). The extracted spat were rinsed and counted.

Table 3 presents the spat density data for the 2 treatments. By day 4, the unnetted treatment had a significantly lower spat density than did the netted treatment (Mann-Whitney U-test statistic=336, d.f.=1, p<0.0005). On day 14, the spat densities were very similar to those found on day 4; there were still significantly more spat on the netted pads than on the unnetted pads (Mann-Whitney U-test statistic=375, d.f.=1, p<0.0005). Over the first 4 days of the experiment, the mean spat density of the unnetted pads was reduced to a density equal to 56% of the netted pads.
Table 3. Mean densities (± S.D.) of *Mytilus edulis* spat (0.5-1.5 mm) on days 4 and 14 of the large scale caging experiment. Comparisons were made using the Mann-Whitney U-test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Spat Density (± S.D.) (no./30 cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>day 4</td>
</tr>
<tr>
<td></td>
<td>$\bar{x}$  n  p</td>
</tr>
<tr>
<td>Net</td>
<td>578 ± 221  20  &lt;0.0005</td>
</tr>
<tr>
<td></td>
<td>571 ± 149  20  &lt;0.0005</td>
</tr>
<tr>
<td>No Net</td>
<td>322 ± 84  20</td>
</tr>
<tr>
<td></td>
<td>306 ± 78  20</td>
</tr>
</tbody>
</table>
This indicated that approximately 17,000 spat were eaten from each unnetted pad during this time. On day 4 I noticed that the clusters of spat that had been present on the pad surface of both treatments had disappeared from only the unnetted pads, leaving just those spat occurring down in the matrix of the pad. Day 4 (August 18) was also the last day that any surfperch were caught from the raft (see the fish trapping section below).

2. Fish Trapping and Observations

A. Seasonal Pattern of Surfperch Abundance.

Figure 11 compares the seasonal pattern of caging effects (Figure 11A) with the seasonal pattern of surfperch abundance (Figure 11B,C) at the raft over a one year period. Figure 11A plots the cage effects for Experiments 8-16. Cage effect was defined as the mean percent mortality from uncaged tiles minus the mean percent mortality from the small mesh cage tiles ($\pm$95% confidence interval of the mean difference). Significant caging effects, as measured by ANOVA ($p<0.05$), occurred in all experiments between May and September, but not in other months.

Figures 11B and 11C give the results of the fish trapping at the raft site during this same period. The data for the most abundant species caught, *Cymatogaster aggregata* (shiner surfperch), are shown in Figure 11B. This species was present between May and August at the raft, the same months that had significant caging effects. The other species caught (4 other surfperch species) showed
Figure 11. A. This figure summarizes the results of the tile caging experiments conducted at the raft between 8/86 and 8/87 (Experiments 8-16). Cage effect was defined as the mean percent lost from uncaged tiles minus the mean percent lost from the small mesh cage tiles (±95% confidence interval of the mean difference). B. Number of shiner surfperch, *Cymatogaster aggregata*, per one hour trap at the raft between 9/86 and 9/87. C. Number of individuals of the other surfperch species caught in the same traps described above. PILE = *Damalichthys vacca*; BLACK = *Embio-toca jacksoni*; DWARF = *Micrometrus minimus*; WALLEYE = *Hyperprosopon argenteum*. 
Figure 11
a similar seasonal abundance pattern (Figure 11C), though they were caught less frequently than was *C. aggregata*. The pile surfperch, *Damalichthys vacca*, was the second most abundant species. Except for *C. aggregata* and the dwarf surfperch, *Micrometrus minimus*, all individuals caught were juveniles (<13 cm total length).

B. Cohort Analysis of *Cymatogaster aggregata*.

The size frequency distributions of *Cymatogaster aggregata* are shown in Figure 12. In May 1987, when this species first appeared, the population was composed solely of larger individuals (>8 cm), and many of these were pregnant females. These larger individuals became rare by July 27, presumably because they migrated away from the shallow, inshore farm environment, after having given birth to the young-of-the-year cohort.

These young-of-the-year were first observed on June 3 and they remained abundant throughout the summer. The appearance of the young cohort in June coincided with the high losses observed under the large mesh cage (Experiment 15, Figure 7). Up until August 5, this young cohort included many individuals less than 6 cm in total length. In contrast, on August 16 and September 4, these smallest size classes were rare. Perhaps this explains why the large mesh cage regained its effectiveness in August (Experiment 16, Figure 7).
Figure 12. Size frequency histograms of the 10 different samples of shiner surfperch, *Cymatogaster aggregata*, caught between May and September, 1987. The y-axis is the percent that each size class contributed to the whole sample. The date that each sample was collected is given in the upper left. The sample size, n, is the total number of fish measured. The July 14 histogram is composed of narrower size classes than are the other histograms. This is because on that date, a size class interval of 0.64 cm (1/4 in.) was used, rather than an interval of 1.0 cm.
Figure 12

<table>
<thead>
<tr>
<th>Date</th>
<th>Count</th>
<th>PERCENT FREQUENCY</th>
<th>TOTAL LENGTH (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5/7</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5/20</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>6/3</td>
<td>62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/19</td>
<td>55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/26</td>
<td>66</td>
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<td>7/14</td>
<td>59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7/27</td>
<td>78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8/5</td>
<td>64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8/18</td>
<td>88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9/4</td>
<td>33</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
C. Gut Contents of Captured Fishes.

Between 4/20/87 and 8/18/87, the gut contents of 65 *Cymatogaster aggregata* and 17 juvenile *Dmalichthys vacca* were examined. Four (6.1%) of the *C. aggregata* contained between 1 and 3 *Mytilus edulis*. The other diet items of the shiner surfperch were caprellid amphipods, tube dwelling gammarid amphipods, fish eggs, and the mussel, *Musculus senhousii*. Seven of the *D. vacca* (41.2%) contained between 2-20 *M. edulis* (2-6 mm). The 20 *M. edulis* found in one *D. vacca* composed about 90% of its medium-full gut. In addition to *M. edulis*, the juvenile pile surfperch were eating compound ascidians, tube dwelling gammarid amphipods, and caprellid amphipods.

On 8/13/88, a school of small surfperch was noticed feeding on a large (approximately 100,000) crop of mussel seed growing on green scour pads that had been planted 6 weeks earlier and which were then 5-10 mm in length. About half of these seed were tagged. A trap was set next to this crop for several hours. One *C. aggregata*, one juvenile black surfperch, *Embiotoca jacksoni*, and one dwarf surfperch, *Micrometrus minimus*, were captured. The dwarf surfperch contained no mussels, the black surfperch contained one *M. edulis* (3 mm), and the shiner surfperch contained 8 *M. edulis* (3-10 mm), composing approximately 70% of its total gut contents. Three of these mussels were tagged. In addition to this visual observation of surf-
perch predation, 5 other such observations were made during the 3 summers of this study.

3. Laboratory Feeding Trials

Figure 13 shows the results of the laboratory feeding trials. *Cymatogaster aggregata* quickly picked off and consumed small mussels from the tiles. For instance, within 30 minutes, the 15 medium (9-11 cm) *C. aggregata* consumed nearly all the 2-4 mm mussels (Figure 13A). During these first 30 minutes, I noticed that the fish concentrated first on the 2-5 mm mussels. After these were gone, they began eating more of the 1, 6 and 7 mm mussels.

By 24 hours, the medium fish usually had stopped feeding on the remaining mussels. However, at these latter hours, an individual occasionally picked off a 6 or 7 mm mussel, held it in its mouth for a few seconds, and then spit it out undamaged, as if it was unable to crack the shell in its jaws. At 30 hours (Figure 13B), the 6 and 7 mm mussels had suffered intermediate levels of mortality, but the 8 mm mussels were left untouched.

The mussel size selection by the large shiner surf-perch (12-14 cm), shown in Figure 13C, was a bell shaped curve similar to that of the medium fish, but shifted to the right by several millimeters. These large individuals preferred 3-6 mm mussels, but also consumed many 7 and 8 mm mussels. Nearly all of the 9 and 10 mm mussels escaped predation, though a few of these were eaten. A few 10 mm mussels were found with cracked shells, but not consumed.
Figure 13. Prey size selection by *Cymatogaster aggregata* feeding on *Mytilus edulis* in the laboratory. Medium fish were 9-11 cm total length while large fish were 12-14 cm total length. There were 14-15 fish together in each trial, and they were presented with 8-10 tiles, each seeded with 39-50 mussels of a different size class.
Figure 13

MEDIUM
0.5 hr

PERCENT EATEN

MEDIUM
30 hrs

LARGE
30 hrs

MUSSEL LENGTH (mm)
In all these feeding trials, clean shell fragments of *Mytilus edulis* began accumulating on the bottom within 1 hour after initiation of the trial. By 3.5 hours, there were many of these clean shell fragments of eaten mussels on the bottom. This rapid accumulation of clean shell fragments indicated that the fish were winnowing and spitting out some of the shell, so that it did not pass through the gut.

**DISCUSSION**

Small surfperch, mainly *Cymatogaster aggregata*, were an important cause of mortality to *Mytilus edulis* planted in Tomales Bay. The seasonal pattern of inshore migration and reproduction of surfperch caused a seasonal pattern of very high summertime mortality to small planted mussels. Spat planted between May and September were likely to be destroyed at both the dock and raft sites, whether they were planted on rough tile, smooth tile, or scour pad material. The predation was often rapid and devastating.

One interesting result was that the large mesh cage temporarily lost its protective ability during mid-summer. At this time only the small mesh cage offered protection to the mussels. Coincident with this result was the appearance of a strong cohort of young-of-the-year surfperch which were observed feeding on the tiles in Experiment 15. Soon after the older cohort departed the farm cove and the young cohort grew significantly, the effectiveness of the
large mesh cage was restored. These correlated observations suggest that a changing age structure of the predator population led to the observed changes in cage effect.

Surfperch predation will probably be important in many other bays along the Pacific coast of North America if summer crops of *Mytilus* spat are attempted. *Cymatogaster aggregata* and *Damasichthys vacca* are broadly distributed, occurring from Todos Santos Bay, Baja California to Port Wrangle, Southern Alaska (Tarp, 1952). *C. aggregata* is also one of the most abundant fish species, whether measured in numbers or biomass, in the bays and estuaries within its range. For instance, it was the second most abundant species in Elkhorn Slough, California (Barry and Cailliet, 1981), Morro Bay, California (Horn, 1980), and Mugu Lagoon, California (Onuf and Quammen, 1983). In the Fraser River Estuary in British Columbia, it was the second to fourth most abundant species (Gordon and Levings, 1984). Though *C. aggregata* has not previously been recognized in the literature as a mussel predator, mussel growers in Puget Sound Washington have observed them feeding on their crops. One mussel grower there has observed schools of *C. aggregata* feeding on the natural settlements of small mussels on his culture ropes. He used protective nets to improve production (Doug Skidmore, Race Lagoon Mussels, pers. comm.).

Netting was also found in the present study to be an effective method for eliminating or reducing fish
predation. While a mesh size of 22 x 22 mm was sometimes satisfactory, as mentioned above, at times it was ineffective, and only the 22 x 11 mm mesh worked. Tubular, plastic netting, such as that used in the large scale caging experiment, is an effective means for protecting large crops of spat. Alternatively, the spat could be reared inside lantern nets (Westcott Bay Sea Farms, Friday Harbor, WA) of 12 mm mesh diameter.

Another possible approach for growers to avoid surfperch predation, besides using netting, is to plant the spat early in the year so that they grow to an invulnerable size by the time these fish move onto the farm habitat. For instance, the March 21, 1988 crop described in Chapter IV grew rapidly and reached a mean size of 11 mm by May 21, which is close to when the shiner surfperch became abundant. The laboratory feeding trials (Figure 13) showed that Mytilus edulis reaches a size refuge against shiner surfperch at approximately 11 mm. It must be cautioned however, that although early planting may be effective against shiner surfperch and other small surfperch, it is ineffective against large pile surfperch, Damalichthys vacca, which readily consume 25 mm Mytilus (Brett, 1979). Unprotected winter crops are also likely to be destroyed by scoter ducks (Mellanitta spp.), as was Crop 3 of Chapter V. If either large surfperch or scoter ducks are likely to be abundant, protective netting is necessary.
Although small surfperch caused much of the observed mortality in this study, there were still significant unexplained losses occurring during the winter and underneath the small mesh cages. What caused these losses? One factor which appeared to be particularly important was dislodgement and drop off of live spat. This was observed in several caging experiments and on many of the commercial crops grown in Marconi Cove (Chapter V). As discussed in Chapter V, drop off was often apparent when a seeded substratum was disturbed, usually by emersion (for inspection or moving) followed by reimmersion. These spat were poorly attached to the substratum and sometimes were crawling on it, and so were susceptible to dislodgement by water currents.

The above observations of spat falling off their substrata support the view expressed by Bayne (1964) and Lane et al. (1985) that post-larval pelagic movement away from the site of prior attachment can be an important ecological factor in the dynamics of mussel populations. However, the present data show that this secondary planktonic movement by 1-2 mm post-larvae is not an obligate part of the life history of mussels; it need not occur at all and usually did not. If planktonic migration by 1-2 mm post-larvae was very prevalent, then mussel aquaculture using hatchery produced 1 mm spat would be impossible. But most uneaten post-larvae remained on the tiles for the 1 month experimental period, usually growing beyond a size
capable of effective drifting behavior. Longer term crops discussed in Chapters IV and V support this view.

Instead of an obligate life history phase, the present study suggests that drop off is highly variable and probably depends on many environmental factors interacting together and with the spat's own behavior. This view is supported by McGrath et al. (1988) who found that larval *M. edulis* often recruited directly onto mature mussel beds in West Ireland, rather than arriving there as post-larvae that had previously settled elsewhere.

**LITERATURE CITED**


IV. DENSITY DEPENDENCE OF GROWTH, FOULING, AND MORTALITY

INTRODUCTION

The density of planting is a crucial variable that affects production of any crop. An initial density that is too high may result in reduced growth and vigor of the crop and a concomitant increase in mortality. This intraspecific competition is probably common in natural mussel populations where recruitment is often very abundant. For instance, Kautsky (1982) measured *Mytilus edulis* densities in the Baltic Sea of up to 158,000/m² and found growth rates to be suppressed relative to less dense populations.

On the other hand, initial densities that are too low will waste valuable space which may become overgrown with fouling organisms. A typical mussel culture rope produces approximately 350 market-sized (70 mm) mussels per meter (Dare and Davies, 1975), which is equal to about 0.4 mussels/cm² of rope surface, assuming a rope of 2.5 cm diameter. If one were to initially seed the rope with this low density of 1 mm spat however, they would only occupy approximately 0.3% of the available space at the time of planting. The large expanses of open space might be usurped by fouling organisms before the *Mytilus* population was big enough to dominate the space.
In Marconi Cove on Tomales Bay, open subtidal hard surfaces are rapidly covered by solitary and compound ascidians during the warmer months (pers. obs.). Fouling is a problem common to mussel growers in many areas, particularly when bare collector ropes are suspended in the water in an attempt to obtain natural settlement of mussel spat. Chaves (1975) found that collector ropes suspended in Seabeck Bay in Puget Sound, WA, developed an overgrowth of fouling organisms which inhibited mussel settlement, unless the ropes were deployed at precisely the time when the mussel larvae were settling. Dare (1980) also cited fouling as a serious obstacle to successful mussel culture in parts of Scotland. Thus at the low end of the density spectrum, interspecific competition is likely to threaten a crop of small mussel spat, whereas at the high end, intraspecific competition threatens it.

A strategy, therefore, needs to be developed by which hatchery reared, small (1-2 mm) spat can be planted while avoiding these threats. There is very little information available on the handling and planting of these small spat, since growers work with naturally settled spat and usually allow them to grow up to an easily observed and handled size, such as 1 cm, before using them (Korringa, 1976). However, Dare and Davies (1975) did transplant small 1-2 mm spat from an area of high natural spatfall to a remote grow-out site. They found a strong density dependence of
mortality in transplanted spat. The lower the initial planting density, the better the survival was to market size. The lowest initial density of 1,400 spat/m of rope (1.75 spat/cm², assuming a rope of 2.5 cm diameter) produced the best results.

In this chapter, I report the results of an analysis of how the initial density of small (1-2 mm) spat influences the growth, mortality and fouling in the crop so that a reasonable strategy can be developed for planting these spat.

MATERIALS AND METHODS

Experiment 1: September Planting

This experiment compared the growth and survival of spat planted in the fall at 3 densities that were either left undisturbed (unweeded treatment) or were periodically weeded to remove fouling organisms (weeded treatment). This weeding treatment was done in order to investigate how the fouling organisms affected the mussels. The initial number of spat in each patch, which is defined below, ranged between 39 and 2,400. This corresponded to a range in densities for the 6 treatments of 0.7-42.8 spat/cm², as shown in Table 1. There were 4 replicate patches for each treatment. The experiment was conducted at Marconi Cove between September 19 and December 3, 1985 (75 days).

The 24 patches consisted of square (7.5 cm on a side) pieces of fire hose material seeded with spat and then attached to a plywood sheet. The seeding was done by
Table 1. Initial and final tagging data for each treatment of Experiment 1.

*=p<0.05, **=p<0.01. LU=low density, unweeded. LW=low density, weeded.
MU = medium density, unweeded. MW = medium density, weeded. HU = high density, unweeded. HW = high density, weeded.

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<th>TRTMNT</th>
<th>DENSITY (cm⁻²)</th>
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<th>x²</th>
<th>p</th>
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</thead>
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<td>RANGE</td>
<td>% TAG</td>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>1.2-1.4</td>
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<td></td>
<td>76.9</td>
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<td>1702</td>
<td>79.4</td>
<td>1895</td>
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</table>
sprinkling light-tagged spat (initial mean length=1.9 mm, S.D.=12.5, n=189) down onto the fire hose in a laboratory tank and allowing the spat to attach for 1 week while ambient sea water flowed through the tank. After this time the spat in each patch were counted. The low and medium density patches were counted directly under a dissecting microscope at 12x power. The high density patches had too many spat to count directly and so the number of spat in these patches was estimated by random quadrat sampling. The 95% confidence intervals of this estimate varied between 5% and 19% of the estimate. A sample of approximately 100 spat was taken haphazardly from each patch and the percent tagged was scored.

The patches were arranged randomly on the inside surface of a shallow plywood box (110 x 75 cm, Figure 1) which was covered with multi-purpose garden netting (Ross Co.) to exclude surfperch. This box holding the 24 patches was floated in a subtidal area of Marconi Cove such that the patches were on the underside of the plywood, facing down.

Four of the 8 patches of each density were randomly chosen to be periodically weeded. Weeding was done on 4 occasions, on days 22, 34, 42, and 55. On these days, the plywood box was taken up on shore and turned upside down and filled with water to protect organisms from desiccation. Then all the visible sessile organisms, except mussels, were picked off from the appropriate patches using a knife and forceps. All the patches were photographed on
Figure 1. Experiment 1 on day 0. Each square piece of fire hose material (7.5 x 7.5 cm) was seeded with either a low, medium, or high density of *Mytilus edulis* spat and was then randomly arranged on the inside surface of a shallow plywood box. The box was covered with netting to exclude surfperch and was floated in Marconi Cove for 75 days.
days 34 and 66. As the mussels grew, the patches expanded in diameter until some of the largest patches began to touch one another. Consequently, on day 49, the plywood backing was cut up into individual squares, each square holding one patch. These squares were floated, approximately 15 cm from each other, within a net pen made of garden netting for the remainder of the experiment. The net pen was needed at this time primarily to exclude scoter ducks (*Mellanita* spp.).

At the termination of the experiment, all the mussels of each patch were removed from their substratum and were counted. In the low density patches, all the lengths of all mussels were measured and examined for tags. This could not be done for the medium and high density patches, which contained too many mussels. Instead, a truly random sample of 100 mussels was selected from each patch using the following method. All the mussels from a patch were lined up into rows of 10 mussels each, so that each mussel was assigned a number. Then a list of 100 random numbers, ranging between 1 and the total number of mussels in that patch was generated using a random number algorithm. The lengths of these 100 mussels were then measured using vernier calipers. These mussels were also examined for the presence or absence of a tag.

**Experiment 2: March Planting**

This experiment also compared the growth and survival of mussels grown at 3 different densities, but this time
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Nalle Plastics Inc., Austin, TX). Each of these seeded pads constituted a "patch". The mean and range of initial densities for the three density treatments are shown in Table 2. These patches were held for 72 hours in 6 tanks (0.3 m x 1.6 m) of sea water located out on the end of the dock which rocked back and forth due to the wind waves. This rocking motion was thought to stimulate firm byssal attachment. After the 72 hours, the tanks were partially drained and were transported by boat out to an empty long line from which the patches were suspended. Wire was used to hang the patches 20 cm down below the long-line. This separation of patch from long-line prevented the mussels from crawling up onto the long-line. The patches were spaced 30-60 cm apart, their order was randomized using a random number table, and they were 15-60 cm below the surface. At spring low tides, the patches were 3 m above the bottom.

This experiment was sampled on days 59 and 101 by randomly selecting 4 patches from each treatment. The mussels from these patches were lined up into rows and were counted. The first 30 mussels from each patch were examined for tags. A random sample of 100 mussels was collected for length measurements, as described above for Experiment 1, from one of the patches from each treatment. In addition to this destructive sampling, 2 patches from each treatment were photographed at approximately 14 day intervals throughout the experiment.
Table 2. Initial and final tagging data for each treatment and sampling date for Experiment 2. On day 0, the samples were taken from the whole mass of spat before it was split up into the 3 different treatments.

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<th>DAY 101</th>
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<td>MEAN</td>
<td>RANGE</td>
<td>% TAG</td>
<td>n</td>
</tr>
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<td>2.1-2.4</td>
<td>95.6</td>
<td>429</td>
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<td>MEDIUM</td>
<td>11.0</td>
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<td>HIGH</td>
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<td>22.4-22.5</td>
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<td>COMBINED</td>
<td></td>
<td></td>
<td>96.8</td>
<td>600</td>
</tr>
</tbody>
</table>
RESULTS

Experiment 1: Fall Planting

Table 1 gives the initial and final percent tagged for each treatment of Experiment 1. When the data from all these treatments were combined, there was no significant change in percent tagged over time. Thus there was no large influx of wild untagged spat into this experiment as a whole. However, in 2 of the treatments, percent tagged did change significantly. The percent tagged in the medium-unweeded treatment (MU) increased, whereas that of the high-weeded treatment (HW) decreased. Two sources of error probably caused these deviations. First, some of the tags were ambiguous in this batch of spat. In other words there was a "gray area" of faint tags which required subjective judgment. Secondly there was variation in percent tagged from sample to sample within any given patch. The sampling on any given patch was not always exhaustive enough to account for this variation. Thus the tagging in this experiment was not of high enough quality to be able to detect an influx of a few wild spat onto an isolated treatment. It did however show that there was no significant influx onto this experiment as a whole.

Figure 1 shows the seeded squares of fire hose of Experiment 1 attached to the plywood backing shortly before being floated in Marconi Cove, seeded side down. Thirty four days later, on October 23, the unweeded patches had
developed varying degrees of fouling, depending on the initial density (Figure 2). The low density patches (Figure 2A) were very fouled, mainly by the beige colored, sheet forming compound ascidian, *Diplosoma macdonaldii*, and the foliose, reddish bryozoan, *Bugula neritina*. The mussels in these low density patches were isolated from one another and were surrounded and partially overgrown by the *D. macdonaldii*. It thus appeared at this time that the low density mussels were seriously threatened by the fouling organisms. In the medium density treatment (Figure 2B), more of the pad space was occupied by *Mytilus edulis* and less by these fouling species. Most of the mussels were aggregated into small, unfoiled patches. At the high density (Figure 2C), all the pad space was monopolized by a mat of mussels which expanded outward and over the sheet of *D. macdonaldii*. The pinkish color of the *D. macdonaldii* in Figure 2C, which surrounds the patch of mussels, is an artifact of the photographic development.

Figure 3 shows 3 of these unweeded patches 32 days later, on November 13. By this time, all the *Diplosoma macdonaldii* had died off, leaving the wooden surfaces surrounding the patches bare. The *Bugula neritina*, however, did not die back, but instead became very abundant in the low density treatment (Figure 3A), so that the mussels underneath were no longer visible. In contrast, the mussels in the medium density patch shown in Figure 3B, which had a density of 8/cm², had by this time taken over nearly
Figure 2. Three unweeded patches from Experiment 1 on day 34. A. Low density. Bar is 2 cm. B. Medium density. Bar is 2 cm. C. High density. Bar is 4 cm.
Figure 3. Three unweeded patches from Experiment 1 on day 66. A. Low density. Bar is 5 cm. This bar applies to B. and C. as well. B. Medium density. C. High density.
all the space on its pad, and fouling was light. The high density (Figure 3C) patches were larger in diameter and were even less fouled than the medium density patches.

Figure 4 compares initial and final *Mytilus edulis* densities of the various patches of Experiment 1 (solid circles). For comparison, the same data are also given for Experiment 2 (open circles), which will be discussed below. The slope of this regression line of initial vs. final density for Experiment 1 was 1.02, indicating that initial and final numbers were approximately equal. In other words, survival was approximately 100% regardless of the starting density. Thus the density of each patch remained nearly constant (except for changes due to spreading out of the patch) over the 75 days of the experiment.

The mean growth rate (± 95% confidence intervals) of the *Mytilus edulis* of each patch over this period is given in Figure 5, as a function of the density of each patch. At high densities, weeding had no discernible effect on growth rate. Growth rate in these high density patches was significantly slower than any of the other treatments (Student t-test; t=7.8, d.f.=928, p<0.001). The low density treatments were of intermediate growth rate. Here, weeding did have a significant effect. The unweeded low density patches grew significantly faster than did the weeded, low density patches (Student t-test; t=2.8, d.f.=380, p<0.01). Thus at low density, the removal of fouling organisms appeared to slow the mussels' growth.
Figure 4. Initial vs. final densities of *Mytilus edulis* for all patches of Experiment 1 (solid circles) and Experiment 2 (open circles). Each point represents one patch.
Figure 5. Mean growth rate (± 95% confidence interval) in shell length of random samples of *Mytilus edulis* grown at different densities in Experiment 1. Each point represents one patch. Open circles are patches weeded of fouling organisms, closed circles are unweeded patches.
The faster growing of these 2 low density groups (LU) still grew more slowly than did the medium density group (Student t-test; t=3.19, d.f.=962, p<0.01). Thus growth in the medium density patches was faster than in any of the other treatments. Within the medium density patches, weeding had no significant effect on growth rates. The weeding treatment therefore only affected the growth of low density mussels, but not of medium or high density mussels.

Figure 6 shows the frequency distributions of final lengths for each of the low, medium, and high density treatments. Weeded and unweeded patches were pooled together for each density treatment to generate this figure. The low (A) and medium (B) density patches had bell shaped distributions, except that the medium density was skewed slightly towards the larger sizes. The final mean length of the medium density mussels was significantly greater than that of the low density mussels (Student t-test; t=5.03, d.f.=1081, p<0.001). The shape of the high density histogram (C) differed from that of the other 2. This high density sample had a bi-modal distribution. When these high density patches were sampled on day 75, they superficially resembled the medium density patches, in that both these treatments consisted of a solid mat of relatively large mussels. However, upon destructive sampling of the high density patches, a large pool of small, tagged mussels was found underneath and in the interstices of this overlying mat of larger mussels.
Figure 6. Length frequency histograms for *Mytilus edulis* on day 75 of Experiment 1. A. Low density (0.7-1.4/cm²). n=382. B. Medium density (4-10/cm²). n=701. C. High density (38-43/cm²). n=811. Each histogram represents all the length measurements pooled from all patches of a given density treatment. The arrows mark the mean lengths.
Experiment 2: March Planting

Table 2 summarizes the tagging data for this experiment. A high percentage (96.8%) of the spat used in this experiment received a good quality tag. There were no significant changes in percent tagged over the 101 days of this experiment in the low density treatment ($X^2=0.2$, d.f.=1, $p>0.10$), the medium density treatment ($X^2=0.01$, d.f.=1, $p>0.10$), nor the high density treatment ($X^2=0.3$, d.f.=1, $p>0.10$), as measured by Chi-square analysis. Percent tagged remained high, between 95% and 98%. Therefore, there was no significant immigration of wild mussels in this experiment.

Figure 7 shows the development of patch 3L, a low density patch. The photos were taken on days 14, 45, and 88. On day 14 (April 5, Figure 7A), the patch was covered by benthic diatoms which obscured the small (3 mm mean length) spat. By May 5 (day 45, Figure 7B) the patch was colonized by filamentous red algae, among which the mussels (9 mm mean length) were growing, though they cannot be seen in this photograph. Figure 7C shows this same patch 43 days later, on June 17. The mussels had grown rapidly and re-arranged themselves on the exterior of the pad, which had folded up into a tubular shape within the net tubing. At this time there was very little fouling and the mussels dominated the space.
Figure 7. The development of patch 3L, a low density (2/cm²) patch of Experiment 2. A. Day 14 (April 5). B. Day 45 (May 5). C. Day 88 (June 17). The paper strip in C. is approximately 10 cm from the left hand edge to the right hand edge.
Figure 7
Figure 8 shows the same time sequence for the medium density patch, 12M. On day 15 (Figure 8A), this patch closely resembled patch 3L (Figure 7A) in that the inconspicuous spat were covered by a benthic diatom mat. However, on day 45 (Figure 8B) this patch was already becoming dominated by mussels which were growing up among filamentous red algae. These mussels went on to completely dominate patch 12M (Figure 8C, day 88), forming a thicker mat, relative to patch 3L, which surrounded the folded up pad upon which they had originally been seeded, and extended part way up the supporting net tubing.

Patch 9H, a high density patch, (Figure 9) was very similar in its development to patch 12M. One difference however was that on day 45, the mussels on the high density patch (Figure 9B) appeared to occupy even more space than they did on the medium density patch at this time (Figure 8B). But by day 88 (Figure 9C) the high density and medium density patches could not be visually differentiated.

The change in mussel density over time in these patches is shown in Figure 10. Mortality was density dependent. The high density patches lost between 79% and 91% of their original mussels. Mortality was less severe in the medium density patches, which lost between 59% and 74% of their original mussels. The low density patches had a much lower mortality rate. These patches lost only between 17% and 35% of their original mussels. Thus, the 3 treatments
Figure 8. The development of patch 12M, a medium density (11/cm²) patch of Experiment 2. A. Day 14 (April 5). B. Day 45 (May 5). C. Day 88 (June 17). The mass of mussels in C. is approximately 32 cm long.
Figure 8
Figure 9. The development of patch 9H, a high density (22/cm²) patch of Experiment 2. A. Day 14 (April 5). B. Day 45 (May 5). C. Day 88 (June 17). The mass of mussels in C. is approximately 32 cm long.
Figure 10. The change in density over time in Experiment 2. Sampling was performed on day 0 (March 21), day 59 (May 19), and day 101 (June 30). Solid triangles = high density; solid circles = medium density; open circles = low density. Each point is the density of one patch.
which started out at very different densities converged
upon each other to a narrow range of final densities. This
result can also be seen in Figure 4 (open circles), in
which the initial and final densities were plotted against
each other.

The curves shown in Figure 10 may be misleading in that
straight lines were used to connect the first and second
sampling dates, suggesting constant mortality rates during
this period. In reality, the mortality may have occurred
in sudden, precipitous events such as when overcrowded
clumps fell to the bottom, or were ripped off by large
fish. On day 34 (April 25), several of these medium and
high density patches were observed to have newly created
(unfouled) patches of open space.

The densities shown in Figure 10 were calculated by
dividing the total number of mussels in a patch by 234 cm²,
which was the area of one side of the scour pad substratum.
The mussels confined themselves to this side through day
45, but after this time, they spread out to both sides, and
up the netting material which held the pad. They also
created their own substratum of byssal mat and crushed
fouling organisms which overlaid the pad material. By day
101, the actual surface area occupied varied between 367
and 444 cm². Thus the surface area occupied by the mussels
nearly doubled over time. Based on these actual final
surface areas, the mussel densities ranged between 1.0/cm²
(low density) and 2.6/cm² (medium and high densities).
Figure 11 shows the growth of mussels in the 3 density treatments. Growth was similar in all treatments. However, on day 59 (mid-May), the low density mussels were significantly larger (Student t-test; t=3.4, d.f.=598, p<0.001) than either the medium or high density mussels which did not differ from each other (Student t-test; t=0.9, d.f.=499, p>0.3). This difference disappeared by day 101, when there were no significant differences in length between the 3 treatments (ANOVA; F=1.3, d.f.=2, 296, p=0.26).

DISCUSSION

The 2 experiments conducted in this study differed markedly in the way in which crowding manifested itself. In Experiment 1, there was no significant mortality of mussels in any of the density treatments and consequently these treatments remained different, and had pronounced effects on the growth of the mussels. In Experiment 2 on the other hand, density had its main effect on mortality. The higher the density, the higher the mortality rate, so that the 3 treatments converged upon each other to a similar, low density.

Why did the 2 experiments differ so much in their response to crowding? The most likely explanation is that the wave and current energy during Experiment 1 was less than it was during Experiment 2. Experiment 1 was conducted in the fall when wind velocities are on average much
Figure 11. The increase in mean shell length (± 95% confidence interval) over time in Experiment 2. Solid triangles = high density; solid circles = medium density; open circles = low density.
less than they are in the spring. In addition, the patches of Experiment 1 were growing on the inner surface of an upside down wooden box, the sides of which must have protected the patches from the direct effect of wind waves. The patches of Experiment 2 on the other hand were unprotected from the spring wind waves which probably encouraged unstable, overcrowded clumps on the medium (11/cm²) and high density (22/cm²) patches to fall off.

This result in Experiment 2 is very similar to that found by Boromthanararat and Deslous-Paoli (1988) for wild mussels on intertidal stakes (bouchots) in France, and by Dare and Davies (1975) in Wales. The latter authors attributed the density dependent losses observed to unstable masses of mussels caused by overcrowding coupled with high current speeds, turbulence and drifting materials. Their lowest mortality rate occurred on the lowest density rope which initially had 1.75 spat/cm². Similarly, the lowest mortality observed in Experiment 2 occurred on the lowest density tested (2/cm²). More work needs to be done to determine if densities below 2/cm² lead to even higher survival.

Although this low density of 2 spat/cm² had the least density dependent mortality and intraspecific competition, it also left much open space for fouling organisms to become established. However, in the March planting (Experiment 2), this did not constitute a problem. At that time, the benthic diatoms and filamentous red algae, which quick-
ly colonized the low density pads, were subsequently overgrown by the mussels, so that by day 88, the Mytilus monopolized all the space. This result is similar to that found by Dayton (1973) on the wave-exposed intertidal zone of Washington State. In that community, newly opened space was first colonized by annual, opportunistic filamentous red and green algae. Eventually, however, this space was overtaken by Mytilus californianus. Similarly, in the present study it was possible to start crops in the spring at a favorable low density of 2/cm² without any discernible fouling problems, because the mussels dominated over the algae.

Thus, planting at low density in March proved to be ideal, since both intra- and interspecific competition were minimized. More work is needed to determine whether this result is repeated from year to year. Furthermore, densities below 2/cm² need to be tested for their ability to resist fouling. The spat planted at such low densities might still dominate over competitors, if planted at the proper time of year. This would reduce the labor requirement on growers, since thinning could be delayed or even abolished. At 2 spat/cm², thinning probably has to be done when the mussels reach 25 mm, since in Experiment 2, all the available primary space was used up at this time.

In contrast to the spring planting, the fall planting (Experiment 1) developed a persistent fouling by Bugula
neritina in the low density patches. In order to prevent this fouling, a higher density (8/cm²) was required than was the case in the spring. Thus, the intensity of interspecific competition for space varied with the season. In the mid-summer, it is probably even more difficult to achieve monocultures of mussels, because dense aggregations of large solitary ascidians (probably Ciona intestinalis) were observed to grow on crops planted at that time, such as crop 13, Chapter V. For this reason, the period between May and August should probably be avoided for planting crops of small spat. Instead, fouling was minimized by planting in the early spring.

Interestingly, Diplosoma macdonaldi, which almost overgrew the low density mussels in Experiment 1, suddenly disappeared in November, and the mussels moved into much of the vacated space. Greene et al. (1983) made similar observations on several fouling communities in Puget Sound. They observed a succession from colonial species, including D. macdonaldi, to solitary species, such as Mytilus edulis. One important cause for this succession was simply the difference in longevity between the two groups. The solitary species outlived the colonial species, which often suffered seasonal mass mortality. Similarly, the patches planted in the fall in the present study were partially "self-weeded" by the sudden demise of the D. macdonaldi. Thus, some of the species, like D. macdonaldi, which initially compete for space with the planted M. edulis spat,
may in the long run be harmless, due to their short life span.

If, however, fouling is a persistent problem, it can be reduced by increasing the planting density. The photographs of Experiment 1 showed this effect; fouling was reduced at medium and high density. There were probably 3 different mechanisms causing this. First, denser populations of mussels simply preempted more of the available primary space, leaving less of this space for fouling species to colonize. The secondary space, that is the shells of the live mussels, effectively resisted fouling. This resistance to fouling may be due to the shell cleaning behavior of M. edulis, described by Theisen (1972), or to avoidance by settling larvae.

Second, the feeding activity of dense populations of mussels may have prevented settlement of fouling organisms. Cowden et al. (1984) showed that Mytilus edulis was an effective and indiscriminate predator of a wide variety of marine invertebrate larvae in laboratory feeding trials.

Third, dense mussel patches, such as the high density patches of Experiment 1, expanded outward and over the surrounding fouling organisms, in a manner reminiscent of sheet forming, colonial organisms. This patch expansion and overgrowth of surrounding organisms has also been described in sabellariid polychaetes (Jackson, 1977). In these ways, increased density, whether caused by a grower's
planting strategy, or by the mussels' own gregarious behavior, improved the competitive ability of the mussels.

How important is it then to take measures, such as the above one of increasing the density, to avoid fouling of seed crops? The present study did not actually detect any detrimental effects of fouling. In fact, low density patches of Experiment 1 which were weeded grew slower than did their unweeded counterparts. This result could be misleading because the weeded and unweeded low density patches actually differed slightly in their initial mussel densities. The unweeded patches had more mussels (1.2-1.4/cm²) than did the weeded patches (0.7-1.0/cm²). This higher density itself may have caused faster growth, as will be discussed below. In addition, the weeding procedure could have disturbed the mussels since removing fouling organisms often caused byssal threads to be severed.

It is possible, though it seems unlikely, that the fouling organisms in Experiment 1 were actually beneficial. One conjecture to explain this effect is that the Diplosoma macdonaldi, which was abundant on the unweeded patches, was releasing its larvae (which are relatively large) upon which the mussels were feeding.

The results of this study thus make fouling appear benign and non-threatening to crops of mussel spat. But it would be premature to make such a generalization since more formidable competitors, such as perhaps the large, solitary ascidians mentioned above, may be encountered by other
growers. The effects of fouling on seed crops of *Mytilus edulis* are complex, depending on the species, the abundance, the growth rates and longevity of the fouling organisms encountered. However, early spring (March) was a good planting season in this study since the mussel crop "got a jump" on the summer fouling community. Even low densities of 2/cm², which require less frequent thinning, had little fouling at this time. Fouling of the fall crop was avoided by using the medium initial density (8/cm²).

This medium density had an additional advantage as well. Mussels grown at this density in Experiment 1 grew 11% faster on average than did the low density mussels. The presence of more neighbors somehow enhanced the growth rate of the mussels. Okamura (1986) obtained a similar result. She found that live *Mytilus edulis* with non-living, model neighbors grew less than mussels with living neighbors. Okamura speculated that this effect could be caused by the coordinated pumping activity of the mussels in the patch, resulting in an increased volume of water available to each mussel. This has been shown for colonies of encrusting bryozoans (Okamura, 1985). This growth enhancement at higher densities may help to explain the adaptive value of the gregarious behavior of mussels. Nevertheless, I do not recommend the medium density (8-10/cm²) unless the crop is watched closely and thinned as soon as crowding is apparent. Experiment 2 showed that
medium density patches, if left unthinned, can incur heavy losses.

High densities (20-40/cm²), were clearly undesirable since they quickly became overcrowded. In Experiment 1, this crowding caused a skewed size class distribution having a preponderance of small, suppressed individuals existing under a mat of relatively few, but large, dominant individuals. Terrestrial plant populations typically respond to crowding in the same way. In these populations, density stress has been shown to cause a hierarchy of a few large, dominant individuals and many suppressed, subordinate individuals (Harper, 1977).

This dominance-suppression hierarchy is probably important in the structuring of natural mussel populations as well. For instance, Suchanek (pers. comm.) found that the size class distribution of natural intertidal beds of *Mytilus californianus* in central California was highly skewed to the small size classes. Furthermore, this size class distribution persisted throughout the year. While this stable size class structure of *M. californianus* could be caused by continuous, year round recruitment, it might also be caused by intraspecific competition resulting in a "seed bank" of older, stunted individuals.

**LITERATURE CITED**

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V. OBSERVATIONS ON THE REMOTE SETTING AND GROW-OUT OF LARGE SCALE CROPS

INTRODUCTION

The term "remote setting" usually refers to the shipment of eyed, competent to metamorphose, oyster larvae through the mail to remote grow-out locations where the growers then allow them to set (metamorphose) onto cultch in tanks. After a suitable period, the cultch, with attached spat, is then planted on the grow-out beds (Jones and Jones, 1983). Mussel culture could also benefit greatly from this type of technology. But with mussels, there is no real advantage to shipping larvae as opposed to spat, since mussel spat, unlike oyster spat, will readily attach to new substrata which they encounter. Because the mussel spat are already through metamorphosis, the percent that successfully attach to the cultch would be expected to be consistently high. This contrasts with oyster larvae which often have a low metamorphosis rate (30-40%) in the remote setting tank (Jones and Jones, 1983).

Thus the shipment and remote setting of mussel spat might provide a simple and very useful tool for controlling and increasing production. Consequently, in 1987, Kuiper Mariculture Inc. began providing mussel growers along the west coast of the U. S. with hatchery produced spat for
remote setting, in order to encourage the development of this process. There is a need for more information on how to best implement the remote setting approach for mussels. Chapter IV showed that small spat could be used with high survival, and that early spring planting at a density of 2 spat/cm² produced the highest yield. Chapter III showed how production could be greatly improved by using netting to exclude fish predators. But how can these results be scaled up to a commercial level of production? What practical materials and methods are needed?

In this study I address these questions by observing 8 different crops which varied in 3 important aspects. These were (1) the duration in the set tank, (2) the substratum material, and (3) intertidal vs. subtidal planting. In order to understand better how the duration in the set tank affected attachment, several laboratory observations were made on the rate of formation of byssal threads by spat. In addition, predation by a polyclad flatworm was investigated because one of the crops was attacked by this predator.

MATERIALS AND METHODS

Table 1 gives specific information concerning spat numbers, sizes, densities and setting materials for the 8 crops started in Tomales Bay. The spat used were produced in the hatchery as described in Chapter I, or were obtained from Kuiper Mariculture Inc., Bayside, CA. They ranged in initial length from 0.26-3.0 mm. The spat density was
Table 1. Listing of crop data.

<table>
<thead>
<tr>
<th>CROP</th>
<th>MONTH PLANTED</th>
<th>INITIAL NUMBER (x1000)</th>
<th>INITIAL LENGTH RANGE (mm)</th>
<th>INITIAL DENSITY (no./cm²)</th>
<th>SETTING MATERIAL</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>11/86</td>
<td>1,700</td>
<td>0.30-0.84</td>
<td>13</td>
<td>plywood sheet</td>
</tr>
<tr>
<td>2</td>
<td>02/87</td>
<td>300</td>
<td>0.5-3.0</td>
<td>-</td>
<td>polypropylene rope</td>
</tr>
<tr>
<td>3</td>
<td>11/87</td>
<td>1,010</td>
<td>0.38-0.82</td>
<td>10</td>
<td>coir (palm fiber) rope</td>
</tr>
<tr>
<td>4</td>
<td>11/87</td>
<td>57</td>
<td>1-3</td>
<td>10</td>
<td>poly matrix in low intertidal</td>
</tr>
<tr>
<td>5</td>
<td>11/87</td>
<td>140</td>
<td>1-2</td>
<td>17</td>
<td>light duty scour pad (LDSP)</td>
</tr>
<tr>
<td>6</td>
<td>03/88</td>
<td>392</td>
<td>0.88-1.76</td>
<td>11.1</td>
<td>med. duty scour pad (MDSP)</td>
</tr>
<tr>
<td>7</td>
<td>03/88</td>
<td>465</td>
<td>0.56-1.88</td>
<td>15.6</td>
<td>MDSP in low to high intertidal</td>
</tr>
<tr>
<td>8</td>
<td>07/88</td>
<td>176</td>
<td>0.48-1.36</td>
<td>16.0</td>
<td>rubberized curled hair (RCH) with MDSP backing.</td>
</tr>
</tbody>
</table>
obtained by dividing the number of spat by the surface area of the one side of the substratum onto which the spat were seeded. This was also done in Chapter IV. It is important to note however that the spat slowly spread themselves out over both the front and back of the substratum so that the actual density may be less than the recorded density, even in the absence of mortality.

For some of the crops, the spat were held in the refrigerator, in a damp cloth inside a plastic bag, at 5°C for up to 48 hours prior to transportation from Bodega Marine Laboratory to Tomales Bay. This did not noticeably affect their attachment or crawling behavior.

1. Estimating Initial Numbers

An important preliminary aspect of this study was to develop a method for accurately estimating large numbers of spat. This was done by wet weight extrapolation. The spat were first cleaned by washing them through a series of 2 or 3 screens (500-1500 μm) so that the spat to be counted were of fairly uniform length, and contained very few dead spat, detritus, or clumps of byssal fibers. For instance, Crop 7 had a mean length of 0.95 mm, a range of 0.56-1.88 mm and a standard deviation of 0.28 mm (n=50). The clean, graded spat were collected in a piece of cloth, blotted with paper towels for about 1 minute to remove excess water, and placed inside a covered beaker to prevent evaporation during sampling. Five to 7 samples of the spat were
taken from throughout the clump and were weighed on an analytical balance. These samples ranged from 0.01-0.5 g, which for 1 mm spat equaled 100-2000 spat. Then the remainder of the whole clump was weighed, usually on a top loading balance. The sampling procedure was done as rapidly as possible (less than 30 min.) to minimize evaporation. Usually 7 samples were needed to make an estimate whose 95% confidence interval was ≤10% of the estimate. Each of the samples was then counted under a dissecting microscope at 12x or 25x power.

The wet weights were then plotted versus spat numbers; the resulting regression line was used to predict the number of spat in the large clump of spat. The variance and confidence intervals of this estimate is given by Neter and Wasserman (1974). The wet weight extrapolation method assumes that the regression line generated using small samples of spat also applies to large clumps of spat far out of the range of the data points. For this assumption to hold, the clump must be homogeneous and the samples must be taken as randomly as possible. On one occasion this assumption was tested by counting all 11,386 spat in a sample weighing 0.14 g. This point fell near the regression line generated by the 4 points near the origin (Figure 1).

Figure 2 shows the regression lines generated for spat of differing mean lengths. The correlation coefficients \( R^2 \) for these different lines ranged from 0.98-0.999. Line 1 in Figure 2 was used to estimate the number of spat
Figure 1. A linear regression of wet weight vs. numbers of a group of *Mytilus edulis* spat (mean length = 308 μm). This was a test of the assumption that the linear regression generated from small samples could accurately predict the number of spat in large samples. Only the 4 points near the origin were used to generate the regression line shown.
Figure 2. Linear regressions of wet weight of clumps of spat vs the number of spat in those clumps for spat of the following 6 different mean lengths: Line 1: 0.45 mm. Line 2: 0.57 mm. Line 3: 0.72 mm. Line 4: 0.95 mm. Line 5: 1.21 mm. Line 6: 1.32 mm.
in 47.35 g. This number came out to be 897,000±285,000 (mean estimate ± 95% confidence interval). Thus in this case the estimate was not very precise. But line 2 gave a more precise estimate. This line was used to estimate the number of spat in 13.5 g which came out to be 219,000±21,000. The precision of the estimate is affected by (1) the goodness of fit of the regression line (R²), (2) the number of data points used to generate the regression line and (3) the distance from these data points out to the estimated point (Neter and Wasserman, 1974).

Figure 3 gives the relationship between the slope of the regression lines in Figure 2, expressed as the number of spat per gram wet weight, and the mean length of the spat. This relationship can be used to quickly estimate the number of spat in clumps of clean graded spat of known mean length, but caution is necessary since differences in water content of the clump and in the size distribution of the spat will cause error.

2. **Setting Tanks**

The tanks originally used for setting were made of fiberglass and were trough-like in shape, 30 cm deep x 30 cm wide x 150 cm long. However, most crops were set in a rectangular 432-liter tank, 60 cm deep x 60 cm wide x 120 cm long. This tank had smooth straight walls against which the setting materials could be packed flush with no spaces through which the spat could fall. In later crops, the
Figure 3. Linear regression of the number of spat per gram wet weight (spat/g) on the mean spat length in millimeters (L). $R^2=0.94$. The equation of this regression line is:

$$\log_{10}(\text{spat/g}) = 5.11 - 1.50(L)$$
tank was placed out on a narrow dock exposed to wind-chop which caused it to rock back and forth, thereby agitating the tank water.

3. **Tank Water Quality**

   The tank was filled by bucket with raw seawater, which was passed through a 300 μm screen to remove debris. No air was pumped into the water in most cases. The water was not changed during the setting procedure except for Crop 1, which received a slow (~1 liter per min), continuous input of raw water. This crop was held for 7 days in the setting tank. No supplemental algal food was added to the setting tank of any crop. Tank water temperature varied from crop to crop, depending on the season. Winter crops were set at temperatures down to 7°C, whereas during the spring the temperature rose to 21°C. Salinity is known to vary widely (5-34 ppt) in the Marconi Cove area, as described in Chapter 1. Unfortunately no salinity data were collected from the setting tanks. No setting was performed however, during the heavy rainfall events when salinity is known to plummet to 5 ppt.

4. **Substratum Materials**

   The substratum materials used are given in Table 1. The plywood sheets (1.3 x 1.3 m) of Crop 1 were floated on the water surface, seeded side down (see Figure 7). The coir rope (Hop Growers Supply Co., Toppenish, WA) of Crop 3, a very rough, hairy rope made from palm fiber, was sold in 136 kg bales. Bundles of 20 to 40 strands were slipped
inside black polyethylene shellfish and bait bag net tubing ("Duronet", Nalle Plastics Inc., Austin, TX; see Figure 5). In later crops, a variety of synthetic scour pad type materials (Loren Products, Lawrence, MA) were used, as listed in Table 1. These materials varied between tightly woven, thin fibered mats such as medium duty green scour pad (MDSP), and light duty white pad (LDSP) to the loosely woven, coarser fibered "poly matrix" material. These materials were cut into strips, 5-15 cm wide which were slipped inside the Duronet tubing. This net tubing often forced the strips of material to bend into a "U" shape which ran down the length of the strip. The concave side of the strip was always seeded since there the spat appeared much more protected from abrasion and fish predation. Crop 8 was set onto 2.5 cm thick rubberized curled hair packing material (Blocksom Co., Michigan City, IN). This material is made from animal hair stuck together into a matrix with a green adhesive.

5. **Remote Setting Procedure**

These materials were rinsed in seawater and one layer was packed tightly into the bottom of the setting tank, leaving no gaps through which spat might fall. The materials were temporarily weighted with stones or iron rods. In the earlier crops, the material was covered with only 3-5 cm of water and the spat were sprinkled carefully over the surface area of the material. This was a very slow proce-
dure. For most crops, a faster seeding method was used. The material was covered with at least 30 cm of water and all the spat needed for a layer were mixed into the water all at once. These spat had been washed through a screen (2 mm) just prior to transport to the farm to break up the clumps which form when spat attach byssally to each other.

At the farm, the aliquot of spat to be seeded onto a layer was measured out by a tablespoon and then was gently spread out over the surface of my hands to further break up clumps. Next I plunged my hands in and vigorously mixed them so that the spat were distributed evenly over the length and width of the tank. The spat were then allowed to rain evenly down onto the underlying material. After 30-60 seconds the weights were removed and a second layer of material was packed in on top of the underlying one, and the process was repeated until the tank was approximately half full of seeded material. The weights were then removed and the material was allowed to float up slightly so that the different layers were no longer compacted against each other, but instead were loosely separated by water spaces. The spat in the tank were then left to attach byssally to the substratum materials. The duration of this attachment or setting period varied between 1.5 hours and 7 days, depending on the crop. In addition to the seeding of the material, a few spat were often added to a small dish of water to observe their activity. Usually they began crawling and attaching within several minutes.
6. **Planting the Seeded Substrata**

After the setting period, I removed the seeded materials from the tanks and suspended them from longlines in either Marconi Cove, or Spenger's Cove (Sacramento Landing). Crops 4 and 7 were grown intertidally. Further details of these 2 crops are given below in the Results section. For most crops, the entire setting tank, half drained for ease of handling, was taken by boat out to the site of planting. This way the spat remained submerged and were not allowed to desiccate during transport. Each strip or bundle of material was then shaken vigorously in the setting tank water before removing it and immersing it in the bay. This caused any unattached or poorly attached spat to fall off into the tank rather than into the bay upon immersion.

7. **Measuring Setting Tank Loss**

This was a measurement of all the spat which failed to attach to the substrata, and instead were left behind in the setting tank after planting. These spat were collected from the tank and were counted either directly or by wet weight extrapolation.

8. **Crop Monitoring**

The grow-out area (described in Chapter 1) at Marconi Cove was visited once per week on average. Spenger's Cove, which was less accessible, was visited once or twice per month while crops were growing there. At these times, the
crops were observed by pulling up the materials for inspection and photography. Growth was measured by taking clumps of the mussels down to the substratum. Mortality measurements were made in Crops 6 and 8. The details of these measurements are given in the Results section for clarity.

A cohort of flatworms settled onto Crop 6. These flatworms were studied in the field and laboratory. The details of the methods used are given in the Results section dealing with this topic.

9. **Byssal Attachment Rate in the Laboratory**

The rate of formation of byssal attachment threads by individual *Mytilus edulis* (0.9-1.5 mm) was measured on 3 occasions in the laboratory. I did this to better understand the attachment process which occurred in the setting tanks. The threads and their attachment plaques, being thin and light in color, are usually very difficult to count. Consequently, attachment was measured on glass slides and dishes. Several spat were placed in Stender dishes (10 ml), or onto microscope slides sitting underwater in a Petri dish. The threads secreted were counted under a dissecting microscope by inverting the slide and viewing through the glass.

**RESULTS AND OBSERVATIONS**

1. **Duration in the Setting Tank**

Figure 4 shows the percent of spat which adhered to the substratum material for crops of varying durations in the setting tank. The percent attached was obtained by sub-
Figure 4. The percent of spat which adhered to the substratum material while in the set tank plotted against the hours spent in the set tank. The data are for the different crops which were remote set at Marco-ni Cove.
tracting the number left behind in the setting tank from the initial number. A very high percent of the spat adhered to the materials regardless of the duration in the setting tank. Figure 5 shows Crop 5, which was held in the setting tank for only 1.5 hours, on days 0 (A) and after approximately 1 month (B). It can be seen that after one month there were still many spat attached and growing. Thus, the spat could attach very quickly to the substratum materials.

Aside from the duration in the setting tank, these crops also varied widely from one another regarding other parameters in the setting tank, such as temperature. Temperature varied between 7°C and 21°C, depending on the crop. Thus attachment in the tanks was very successful over a wide temperature range. Attachment also occurred in the absence of aeration, and on a variety of substratum materials which are discussed below in more detail. It is important to note that Figure 4 gives the percent attached after the materials were shaken under water and then pulled out of the tank. It does not tell how many stayed attached or how firmly attached the spat were. On several occasions, spat were observed falling off their substratum at the time of planting.

2. Byssal Attachment Rate in the Laboratory

Figure 6 shows the number of byssal attachment threads produced by 0.9-1.5 mm spat on glass slides and dishes
Figure 5. Two close up views of Crop 5, which had a 1.5 hour set tank duration. On day 0 (A) the 1-2 mm spat were visible as small brown dots on the light duty scour pad. The bar is 2.0 cm. After 1 month (B), many spat were still visible in the material, and had grown to 3-4 mm. The bar is 2.0 cm.
Figure 6. The number of byssal attachment threads produced by individual *Mytilus edulis* spat (0.9-1.5 mm) attaching to glass slides or dishes over various time periods at room temperature (16-20°C). The different symbols denote independent trials using different groups of spat.
Figure 6
after varying durations in the laboratory at room temperature. During the first 43 hours they secreted threads at a mean rate of 9.7 threads per day (S.D.=4.0, n=11). After this point the rate of secretion decreased, though they did not stop secreting threads, since after 408 hours (17 days) the spat averaged 59 threads each. Thus the spat became more and more firmly attached as the days progressed.

3. Substratum Materials

The crop set directly onto sheets of plywood (Crop 1) is shown in Figure 7. The plywood had the advantage that it provided a wide surface area over which the spat could easily be evenly distributed. However the sheets had to be pinned under water in the setting tank due to their buoyancy. Also, the relatively smooth surface of the plywood encouraged crawling of the small spat which formed clumps, only poorly attached to the wood. Nevertheless, most of the spat did attach after 7 days in the tank. The plywood was bulky and difficult to handle during planting, when the sheets were turned over and set on the water surface, seeded side down. In Figure 7B, a bare patch is visible where the seed mussels were scraped away, most likely by chafing against a rope. The relatively smooth texture of the plywood left the spat exposed and vulnerable to such risks.

The dense crop which developed on these seeded sheets contrasted with the 3 unseeded control sheets which accumulated no natural set of mussels. The growth of this crop
Figure 7. A. Overview of Crop 1, the plywood sheet crop, in which the sheets were attached to a double long-line at Spenger's Cove on the western shore of Tomales Bay. B. The underside of one sheet after 4 months (March, 1987).
of seed is shown in Figure 8. Since this crop was planted in November, it grew slowly at first during the cool winter months, but growth rate increased rapidly as spring approached. This crop suffered some losses due to a storm which flipped some of the sheets of plywood upside down.

Crop 2 was grown by Mr. Frank Spenger on the west shore of Tomales Bay, at Spenger's Cove. This crop was grown on discarded polypropylene rope remnants that were old, frayed and knotted. In February 1987, these were tied into loose bundles, placed in a tank, and approximately 300,000 spat, (0.5-3.0 mm) were sprinkled in (Figure 9A). The ropes near the surface received a poor set since the spat quickly sank and fell through the interstices between the ropes. In order to encourage more attachment on the ropes and less on the tank bottom, an oar was used to overturn the mass of ropes, several times per day. The overturning mass of ropes rubbed along the bottom, ensnaring many of the spat on the bottom. After several days the ropes were suspended from a raft in a relatively protected part of the bay. By June, some of the ropes developed thick mats of seed mus-sels (Figure 9B).

Crops 4-8 were grown on synthetic scour pad type mate-
rials. One commonly used material was medium duty green scour pad (MDSP), cut into strips 5-15 cm wide and 4-6 feet long. The strips were slipped inside net tubing. These flat strips could be packed into a tank bottom with almost
Figure 8. Growth of Crop 1. Points are the mean shell length and bars are the range.
Figure 9. Crop 2, during (A) addition of spat to the set tank in February, 1987 and (B) 4 months later, in June, 1987. This crop had been grown from a raft at Spenger's Cove.
no gaps where spat could fall through to the bottom.
Small, unclumped spat (<2 mm) sprinkled into water over a
layer of this material fell down into its fibrous matrix,
but not all the way through it (Figure 10A). The matrix
provided crevices for these small spat. It appeared that
the spat did not crawl far from where they landed, since
the spat did not clump and "ball up" as often occurred on
smooth surfaces. However, large spat (>3 mm), which did
not fit into the interstices of the material, tended to
remain on the surface of the material and there formed
unstable clumps. Thus this material was best suited for
small (<2 mm) spat.

As these spat grew, they migrated outward from the
scour pad matrix (Figure 10B). Figure 11 shows several
MDSP strips which developed into dense aggregations of seed
mussels.

The scour pad type materials are available in a variety
of weaves, some more open and loose than others. One of
these more open weaves, known as "poly matrix", was used in
Crop 4 (described below in the section on intertidal plant-
ing). This material provided a deeper habitat than did
MDSP and it allowed larger spat (2-5 mm) to fit down in the
matrix.

In addition to these synthetic materials, palm fiber
(coir) rope (≈8 mm diameter) was used in Crop 3. Figure 12
shows how it was bundled inside net tubing. This created a
deep, fibrous, complex habitat for the attachment of spat.
Figure 10. A. Medium duty scour pad (MDSP) with newly attached, 1 mm spat. The reddish object at the top of the figure is a ruler, the markings on which are 1 mm apart. B. After approximately 1 month (July/August) in the sea, the spat on this MDSP pad had reached 3-8 mm and had become darkly pigmented. The object in upper right is a 25 cent piece (US). The bar in the upper right is 10 mm.
Figure 10
Figure 11. Four strips of MDSP each with a thick covering of seed which began as small (≈1 mm) spat in the MDSP matrix.
Figure 12. Close-up view of a coir rope substratum, which consisted of approximately 40 strands of coir (1.3 m in length) slipped inside a length of net tubing. This substratum had been seeded the previous day with 2 mm spat. The white object is a ruler. The bar in the upper right is 20 mm.
Crop 3 did grow into a heavy seed crop, but it was largely destroyed by scoter duck predation in February, 1988, before it was photographed.

Crop 8 utilized another substratum, rubberized curled hair packing material (RCH), in combination with MDSP. A strip of RCH was laid on top of a strip of MDSP and this pair of strips was slipped inside net tubing (Figure 13). The RCH has a very porous, open weave. For this reason the strips were laid in the setting tank with the RCH facing upward. When seeding these strips, the sinking spat fell through the RCH and came to rest on or near the MDSP backing. Thus between the spat and the open water was a protective, fibrous layer created by the RCH pad.

The 176,000 spat of Crop 8 were planted on 5 of these strips in July, 1988. As the spat grew, they slowly migrated outward through the RCH material. The substrata was also colonized by many large, solitary ascidians, probably Ciona intestinalis, and by the arborescent bryozoan, Bugula neritina. On September 17, this fouling was largely killed off by drying the substrata in the hot sun for 3 hours and by manually crushing many of the ascidians. On November 14 (day 118), the substrata had a thick covering of seed mussels of mean length 15.9 mm (S.D.=7, n=100) with not much fouling (Figure 14A). This contrasted with the 3 control strips, 2 of which are shown alongside a seeded strip in Figure 14B. The 3 control strips had a total of 11 mussels on them. In contrast, the mass of mussels on
Figure 13. Close-up side view of one of the substrata of Crop 8 lying on a dock on the day of initial planting. The darker green MDSP backing is visible below the lighter green rubberized curled hair (RCH). Both materials are inside a length of net tubing.
Figure 14. A. Crop 8 on day 118, after being pulled from the water and laid on a dock. The mussels are 15.9 mm in mean length. The pinkish objects among the mussels are caprellid amphipods. B. One of the above substrata on day 118 lying next to two unseeded, control substrata, which were dominated by the foliose bryozoan, Bugula neritina.
the 5 seeded strips weighed a total of 20.2 kg whole wet weight and contained 40,824 ± 13,536 (95% confidence interval) mussels. This is 23.1% of the initial number. Thus despite the good appearance of this crop, the density decreased from 16 spat/cm² to 5.2 mussels/cm² over the 118 day period. The cause of this mortality was not determined, but detachment due to crowding was a likely factor.

4. Intertidal Planting

Crops 4 and 7 were grown intertidally on oyster culture racks made of steel rod which suspended the strips 0.3 m above the bottom. Crop 7 was battered by waves and some of the seeded strips were lost and the others had very high losses, apparently due to desiccation and the physical force of the waves. It was situated on the south end of Marconi Cove, facing the north-west wind-waves head on, between +3 ft and -1 ft in the intertidal. Crop 4, on the other hand, was located in the most protected part of Marconi Cove at a tidal level of approximately 0 feet. This crop was seeded onto one side of squares of "poly matrix" material inside net sock which were attached to a rack, seeded side facing down (Figure 15A). It was planted in November 1987, and by February 1988 the lower seeded side had developed into a dense seed crop (Figure 15B).

5. Flatworm Predation

Crop 6 was suspended from a long line in Marconi Cove on 3/3/88 and by 4/1/88 the strips had a thick crop of seed
Figure 15.  A. Crop 4 during a spring low tide in February 1988, 3 months after planting. B. Close-up on the same day of the underside of one of the "poly matrix" pads.
mussels growing out from the MDSP pads (Figure 16A), although I did notice some bare patches at this time. On 4/13 this crop looked poor, with many bare patches. Upon closer inspection on 4/15, many reddish brown polyclad flatworms were found on the crop, as well as many empty mussel shells (Figure 16B). This flatworm was also found on 5 other crops at this time.

Dr. John Holleman (Gavilan College, Gilroy, CA) identified this flatworm as Notoplana inquieta (Heath and McGregor, 1912). The flatworms were mainly clustered around and underneath the remaining clumps of mussels. One of these flatworms with a full gut was taken from such a clump and placed into a jar of seawater where it regurgitated its gut contents. This was the soft tissue of a mussel with the mantle and foot still intact.

Five of the 20 MDSP strips that comprised this crop were haphazardly selected on 4/15 and the number of N. inquieta, and of M. edulis, both empty shells and live mussels, were counted for each strip. Figure 17 shows this flatworm abundance data plotted against the empty shell data for each of the 5 strips. There was a positive correlation ($R^2=0.77$) between the number of empty shells per strip and the number of flatworms on that strip. On these 5 strips, there were a total of 252 N. inquieta (10-28 mm in length), and 5737 M.edulis (5-12 mm), 1440 (25.1%) of which were dead. These dead M.edulis were empty, but undamaged, gaping shells. The inner surfaces of some of
Figure 16.  A. Crop 6 on 4/1/88, 29 days after planting. This is a close-up view of one of the substrata, which consisted of medium duty scrub pad (MDSP) inside net tubing. The mussel seed were covering most of the substrata at this time. B. Close-up view of one of the substrata of the same crop 2 weeks later, on 4/15/88. The net tubing was removed for clearer observation of the flatworms, Notoplana inquieta, (brownish material) and the mussel seed, many of which can be seen to be empty shells.
Figure 17. The number of *Notoplana inquieta* on each of the 5 sampled substrata from Crop 6 on 4/15, plotted against the number of intact, but empty mussel shells on each of those substrata.
these shells were covered with the egg masses of *N. inquieta*. These egg masses were recognized by their identical appearance to egg masses laid by *N. inquieta* in the laboratory. Finding intact but empty shells of seed mussels was generally very rare except in association with this flatworm infestation in April 1988.

I further investigated the importance of *Notoplana inquieta* as a mussel predator by comparing the mussel mortality of 2 crops, one having a high flatworm density and the other having a low flatworm density. The high flatworm density crop was Crop 6, described above. The low flatworm density crop was Experiment 2 of Chapter IV. The medium density treatment of this experiment (11.0/cm²) was similar in initial density to Crop 6 (11.1/cm²). Both crops were planted on the same long line. In addition, the 2 crops used the same substratum material (MDSP). The low flatworm crop was planted 18 days after the high flatworm crop was planted.

I examined the low flatworm crop for empty shells and flatworms on 4/25, 5/5, and 5/11 by pulling the strips from the water and closely searching among the seed mussels, but without dislodging these mussels. Neither empty shells nor flatworms were found until 5/11 when just one flatworm was found. I removed this flatworm. On the final sampling day (6/30) there were no flatworms present on any of the 3 strips sampled. In contrast, when the high flatworm crop
was sampled on 4/15, the mean flatworm density was 50.4 worms/strip (S.D.=32.6, n=5). Thus, these 2 similar crops provided a serendipitous comparison of high vs. low flatworm infestations.

Mortality in the low flatworm crop was measured on 5/20 and 6/30 by randomly selecting 4 replicate strips, as described in Chapter IV. In Figure 18, this mortality rate is compared with that of the high flatworm crop (crop 6). Mortality in the high flatworm crop was extremely high; 96% of the mussels were dead by 4/15, when this crop was sampled. This was equal to a mean mortality rate of 24.7 spat/100 cm²/day (Standard Error=0.9, n=6).

Mortality in the low flatworm crop, on the other hand, was less severe, and could be divided into 2 time periods (Figure 18, solid circles). The early period (3/21-5/20) was the time between the first and second samplings. This early period overlapped in time with the high flatworm crop. Thus the early period is the relevant time period for comparison with the high flatworm crop. During this early period, the spat disappeared from the low flatworm crop at a mean rate of 11.4 spat/100 cm²/day (S.E.=1.42, n=12). This mortality rate was less than half (0.46) that of the high flatworm crop. These two mortality rates differed significantly from each other (Student t-test; t=4.1, d.f.=14, p<0.01).

In addition to the above field study, the feeding behavior of Notoplana inquieta was studied in the laborato-
Figure 18. The decrease in mussel spat density over time in Crop 6, the high flatworm crop (open circles), and the medium density of Experiment 2 of Chapter IV, the low flatworm crop (solid circles). Both crops were grown on the same long-line and used the same substratum material.
ry as follows. Four 1-liter beakers of ambient (9-14°C) seawater were stocked with 400 seed mussels (2-13 mm) each. Two of the beakers received 20 _N. inquiesta_ (10-28 mm long) each. Both the flatworms and the mussels were taken from Crop 6 and were representative of the size range of mussels and worms on this crop. The beakers were aerated, water was changed every 2-3 days, and the mussels were fed _Isochrysis galbana_ (T-ISO) every 1-2 days at 100-200 cells/μl. The number of empty shells found in each beaker was counted on days 0, 2, 9, and 20.

Figure 19 shows the cumulative mortality of mussels in these beakers. The _N. inquiesta_ steadily preyed on the mussels provided to them, while mortality in the control beakers was negligible. Under these conditions, the flatworms consumed 0.6 mussels/day/flatworm on average. Most of the mussels in the beakers arranged themselves into clumps as is usual. The _N. inquiesta_ generally fed on mussels at the base of these clumps. This destabilized the clumps since those mussels attached to the substratum were the first attacked. Whole clumps sometimes fell from the beakers' walls where they had been attached, taking flatworms with them to the bottom.

Because the flatworms were usually hidden among the clumps of mussels, it was difficult to observe their mechanism for penetrating and consuming their prey. I did however observe one _N. inquiesta_ attacking a lone mussel
Figure 19. The cumulative mortality, as measured by the numbers of empty shells found, of four populations of 400 *Mytilus edulis* spat (2-13 mm) each, held in beakers at 9-14°C over a 20 day period in the laboratory. The two experimental beakers (solid triangles) also contained 20 *Notoplana inquieta* (10-28 mm in length) each, while the control beakers (solid circles) had no flatworms.
attached to the wall of the beaker. The flatworm's pharynx was everted out of its mouth and penetrated between the mussel's valves, which were slightly agape.

The *N. inquieta* preferentially selected larger mussels out of those available during the study. The mean length of eaten mussels (8.3 mm, S.D.=1.4, n=90) was greater than those not eaten (7.0 mm, S.D.=2.5, n=199; Student t-test; t=4.8, d.f.=287, p<0.001). The largest mussel eaten was 11.9 mm.

After conducting the above studies, I investigated the control of this flatworm using fresh water as follows. Eight *N. inquieta* were placed into 2 dishes (500 ml) of seawater, 4 worms per dish. All worms attached themselves to the dish surfaces and appeared healthy, as evidenced by their gliding motion. One dish (control) was drained, refilled with seawater and after 1 minute was drained again and refilled with seawater. This was done to simulate the draining and filling of the experimental dish. The experimental dish was drained and then was filled with fresh tap water. After 30 seconds this was quickly drained off and the dish was refilled with seawater.

Immediately upon immersion in freshwater the *N. inquieta* writhed and contorted their bodies. Within 1 hour all 4 worms exposed to the 30 second freshwater dip were dead and detached from the substratum. Their epithelia dissociated from the underlying tissue, and the worms looked as if they were falling apart. All control worms remained healthy.
A fresh water dip treatment was field-tested on a crop of seed mussels belonging to a private grower in Marconi Cove. This crop supported a large population of *N. inquisitata*. The mussels were growing on scour pad inside an oyster grow-out bag made of 18 mm (3/4 in.) mesh diameter plastic mesh. Four of these bags were immersed, one at a time, into a 1.2 x 0.6 m tank of fresh tap water for 3 minutes. The bags were gently swished up and down in the tank to aid in freshwater penetration into the mat of mussels. After the 3 minute dip the bags were examined for worms. Several worms were observed and these all had dissociating epithelia and appeared to be dead.

**DISCUSSION**

1. **The Setting Tank**

   The present study showed that crops of mussels could be successfully started with little manipulation or control of the water quality in the setting tank. Neither aeration, water changes, nor feedings were necessary, and the temperature could range between 7-21°C. In addition, crop 5 showed that it is possible to use only a 1.5 hour duration in the setting tank. Thus the remote setting procedure is flexible and can be very quick, easy and inexpensive.

   Though the feasibility of this setting technique is clear, I did not determine the optimum setting tank conditions and durations for maximizing the yield of mussels. These will likely depend on the substratum used and the
environment into which the spat are planted. In some environments, short term (less than one day), static, unaerated setting tanks may be adequate. In other environments, longer durations with aeration and flowing water may be needed to ensure the firmest possible initial attachment. More research is needed in this area.

The number of byssal threads, and thus the strength of attachment, that each spat possesses can be increased, if needed, in several ways. First, the duration in the setting tank can simply be increased. It was shown in the laboratory that the spat accumulated more and more byssal attachments as the hours and days progressed. After 3 days at room temperature (16-20°C) these spat produced on average 30 byssal threads each, which appeared to provide a secure attachment. Experiment 2 of Chapter IV utilized a 3 day duration in the setting tank, and the low density treatment had high (65-83%) survival to day 101. Thus a setting tank duration of 3 days is reasonable.

Another parameter which will encourage firm attachment is water agitation. Mussels which are exposed to currents and waves attach more firmly than do mussels in quiescent water. For instance, Harger (1970) measured the force required to remove mussels (Mytilus edulis and M. californianus) from calm laboratory aquaria and from the open shore. The force required for any given size mussel was much less in the aquaria than on the open shore. Therefore in the setting tank environment, agitation of the water
should improve the attachment of the spat. This agitation should not be applied immediately upon addition of the spat to the tank however, as this can cause many spat to fall off the substrata to the bottom before they have a chance to attach. Instead it should be applied about 1-2 hours after addition of the spat, so that they have time for initial attachment. Vigorous aeration can be used, as can periodic shaking or stirring of the substrata. Simply situating the tank on a boat or dock which is rocking in the waves may also provide a steady gentle agitation.

2. Substratum Materials

*Mytilus edulis* spat will attach and grow on a wide variety of materials. Smooth surfaces, such as plywood, should be avoided, however, because the spat are exposed and thus more vulnerable to physical abrasion as well as predation. In addition, spat crawl more on smooth surfaces than on rough surfaces. This crawling, in combination with their gregariousness, results in the formation of unstable clumps which can fall off. Maas Geesteranus (1942) found that *M. edulis* were equally attracted to groups of stones, with their angles and hollows, as they were to groups of conspecifics. Thus clumping should be less when the substratum contains such angles and hollows. My observations confirm this view. For these reasons a complex rough substratum is best.

The knotted polypropylene rope bundles used in Crop 2
worked well, probably because of the numerous crevices provided by the knots as well as the black color of the rope which would help to hide the spat from fishes. In addition, these rope fragments were very inexpensive and easy to come by. The only drawback of this material was that it was difficult to seed evenly, since the spat tended to fall through the spaces between the ropes.

In contrast, the strips of scour pad material were well suited to efficient seeding, since they could be packed into the tank bottom, lining the entire bottom. These materials can produce crops with high survival, as seen in the low density treatment of Experiment 2 of Chapter IV. However, the medium duty pad (MDSP), while providing protection for small spat (0.5-2.0 mm), does not provide much protection for larger spat. As the small spat grow, they crawl out of the weave to the surface of the substratum. This problem was overcome in Crop 8 which utilized a layer of rubberized curled hair (RCH) pad as a habitat for the spat as they grew out of the MDSP. This crop had a 525 fold increase in biomass over 118 days. Dare et al. (1983) used a similar rubberized curled hair material known as 'Hairlok' for the collection of wild spat in England with excellent results. Like this RCH material, the coir rope bundles also provided a very protective habitat. Mats made of coir have been used successfully in China (Nie et al., 1979) as well.
3. Flatworm Predation

The observations suggest that *Notoplana inquieta* was an important predator in the spat culturing system during the spring of 1988. These flatworms appeared on 6 different crops at this time and were observed preying on the spat in the field, and they readily consumed healthy mussels in the laboratory. When Crop 6, the high density flatworm crop, was sampled, 25% of the counted mussels consisted of dead, empty shelled individuals. This indicated that the flatworms were causing significant mortality. In addition, this crop had over twice the mortality rate of the low flatworm crop, which served as a control. Based on laboratory observations, the flatworms probably caused losses by destabilization of clumps, as well as by direct consumption. Thus *N. inquieta* is definitely a predator on *Mytilus edulis* seedlings and appears to have played an important role in the high mortality observed in Crop 6.

*Notoplana inquieta* is a native species to the west coast, and has been collected between Los Angeles, California and Deadman's Island, British Columbia (Hyman, 1953). Little is known about the ecology of this species. Hurley (1975) found that *N. inquieta* and another polyclad turbellarian, *Stylochus tripartitus* (which was difficult to distinguish from *N. inquieta*), caused 60-100% mortality to populations of the barnacle, *Balanus pacificus*, on settling plates suspended above the bottom offshore from La Jolla,
California in 2 successive years. This supports the view that \textit{N. inquieta} can be an important predator. This species has not previously been known to feed on bivalved mollusks. However, it is not surprising that \textit{N. inquieta} does feed on mussels. Pelecypods are an important prey item to many polyclad turbellarians such as the oyster predators, \textit{Pseudostylochus ostreophagus} (Woelke, 1957), \textit{Stylochus ellipticus} (Loosanoff, 1956), and \textit{S. inimicus} (Pearse and Wharton, 1938).

While the effect of flatworms on oyster populations has received attention, their effect on mussel populations has not. Several observations by other biologists and aquaculturists suggest however that they may have widespread importance to \textit{Mytilus edulis} populations. For instance, Dr. Mark Page (pers. comm.) found that a flatworm, probably \textit{Notoplana acticola}, preyed on small (<11.8 mm) \textit{M. edulis} among the colonies of these mussels growing on an offshore oil platform in Santa Barbara, California. In Puget Sound, Washington, Mr. Charles Stevens of Kamilche Sea Farms, Olympia, WA (pers. comm.) observed flatworms preying on small \textit{M. edulis} growing on a suspended culture system. Therefore there is probably a variety of polyclad turbellarian species, many of them very similar in appearance, which prey on \textit{Mytilus} populations. More research is needed on these little known mussel predators.

\textit{Notoplana inquieta} apparently recruits onto suspended, off-bottom substrata via a planktonic larva, though this
has never been proven. Probably the easiest way to control this flatworm is to take advantage of its vulnerability to fresh water. Unlike *Stylochus ellipticus*, which is very resistant to low salinities (pers. obs.), *Notoplena inquieta* can be rapidly killed with a 3 minute freshwater dip which will not harm the mussels.

4. Detachment of Spat

Several crops suffered noticeable losses of spat immediately upon immersion into the sea, even though these spat had been attached just moments before in the set tank. This can be a serious problem. Mr. Richard Glenn (Sea Farms West, Carlsbad, CA; pers. comm.) had difficulty using hatchery reared spat because of detachment. Mr. Charles Stevens (Kamilche Sea Farms, Olympia, WA; pers. comm.) has also observed "thousands" falling off upon immersion. Nie et al. (1979) also noted that detachment of spat occurred.

The causes of behavioral detachment of spat are not known. The initial size of the spat may play a role. Blok and Tan-Mass (1977) found that *Mytilus edulis* spat of 0.24-2.0 mm length often migrated planktonically, and Bayne (1964) showed that 1.0 mm spat were the most likely to disperse planktonically. Though this drifting behavior is most prevalent in these small spat, larger spat (2–5 mm) are also quite susceptible to falling off their substrata and sinking to the bottom. On several occasions I observed these larger spat falling off upon immersion or disturbance
of their substratum. Thus size alone is not a safeguard against detachment. And conversely, the small spat (<1 mm) are by no means destined to detach and disperse planktonically. Spat which were less than 1 mm were used routinely in the caging experiments of Chapter III with often high survival and in Experiment 1 of Chapter IV which had nearly 100% survival.

One possible explanation for the observed fall-off of both small and larger spat is that some disturbance, stress or shock associated with removing the substratum and immersing it in the sea causes some of the spat to sever their byssus and perhaps to begin crawling. In this unmoored state they would be susceptible to being swept away by water currents. The specific stress may be a sudden change in temperature or orientation to gravity or light. Desiccation is also a likely cause. More study needs to be done in this area. However, the risk of fall-off can probably be minimized by observing the following guidelines:

(1) Use a protective substratum such as coir rope inside net tubing, or MDSP with a covering of RCH pad. The netting will also protect the spat from fish predators, as discussed in Chapter III.

(2) Use a low density of 1-2 spat/cm² to avoid overcrowding which can create unstable clumps (see Chapter IV). Plant these low density crops in the early spring when fouling is not intense.
(3) Encourage firm attachment in the setting tank. This can be done by giving the spat at least 72 hours to attach, and by agitating the water (with aeration for instance).

(4) Avoid chafing, desiccation, and temperature shock during planting.

(5) If the substrata are planted intertidally, avoid battering by waves.

LITERATURE CITED


CONCLUSIONS

1. Metamorphosis

A 400 l recirculating conical tank, equipped with a downwelling chamber, and stocked with filamentous red algae, such as Polysiphonia pacifica, produced a mean number of 0.95 million post-larvae (500 μm) when stocked with 1-3 million competent pediveligers. This system was thus a reliable method for producing these post-larvae.

2. Tagging

Shell pigmentation in post-larval (<1.5 mm) Mytilus edulis could be turned on and off by varying the ambient light intensity. A 5 day exposure to bright light (> 12,000 lux) followed by 5-10 days of darkness produced a distinctive and persistent band which allowed large numbers of these hatchery reared spat to be distinguished from wild spat.

3. Counting Spat

Numbers of M. edulis spat of given mean length (mm) can be estimated by wet weight using the formula:

$$\log_{10}(\text{spat/g}) = 5.11 - 1.50(L)$$

Where spat/g = the number of spat per gram wet weight, and

L = mean spat length (mm).

4. Fish Predation

The shiner surfperch, C. aggregata, and to a lesser extent, juvenile pile surfperch, Damalichthys vacca, were an important cause of mortality to Mytilus edulis planted in Tomales Bay. The seasonal pattern of inshore migration
and reproduction of these surfperch caused a seasonal pattern of very high (often > 90%) summertime mortality to small planted mussels. Spat planted between May and September were likely to be destroyed at both the dock and raft sites, whether they were planted on rough tile, smooth tile, or scour pad material. Netting (11 mm mesh diameter) was an effective method for preventing this fish predation. In addition, *Mytilus edulis* reached a size refuge against shiner surfperch at 11 mm.

5. Flatworm Predation

*Notoplana inquieta* was an important predator in one year of the study, setting densely on several crops and feeding on healthy 2-13 mm *M. edulis*. Thus, crops need to be observed closely for flatworms. *N. inquieta* was susceptible to osmotic shock; they were rapidly killed with a 3 minute dip in fresh water.

6. Density

Mortality was density dependent. This was probably due to the instability of overcrowded mats of mussels. The lowest mortality (mean=27% after 101 days) occurred at the lowest initial density (2/cm²) tested.

Growth rate also was affected by density. High density (41/cm²) caused a preponderance of small, suppressed individuals existing under a mat of relatively few, but large, dominant individuals. Medium density (8/cm²) caused more rapid growth than either low or high densities.
Fouling was inversely density dependent, and could be controlled by using the medium mussel density (8/cm²). Furthermore, the intensity of fouling varied seasonally. The March crop was only fouled by filamentous red and green algae over which even the low density (2/cm²) mussels quickly dominated. Thus planting in March at low density gave the overall best results.

7. **Remote Setting**

*M. edulis* spat could consistently be shipped and remotely set onto a wide variety of substrata with a high (>90%) percent attachment in the setting tank. The best results were obtained when using complex, deep and fibrous substrata, such as rubberized curled hair material with a scour pad backing.

It was possible to use short term (1.5 hr.), unaerated, static setting tanks. Water temperature could vary widely (7-21°C). However, the best setting tank conditions were not determined. Previously attached spat sometimes fell off of their substratum soon after planting in the sea. The firmness and permanence of attachment was thus variable, and needs more study. To encourage a firm, lasting attachment, I recommend agitating the setting tank water, allowing 3 days for attachment, and avoiding chafing, desiccation, and temperature shock during planting.