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Synopsis of Culture Methods for Marine Fish Larvae

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THE objective of this paper is to provide a synopsis of present technology for small-scale laboratory culture of marine fish larvae. The technology of marine fish culture is relevant to this book because it is one of the best ways to obtain a taxonomic series. "Ahlie" Ahlstrom was a strong proponent of this approach and I lectured on the subject at his request for his courses on larval fish systematics. Marine fish culture has often been reviewed (May, 1970; Houde, 1972a; Houde and Taniguchi, 1979; Shelbourne, 1964; Kinne, 1977) and many additional references may be found in the previous reviews. The key feature of my review is that it is a condensed practical guide and key to the literature for beginners interested in small-scale laboratory culture of marine fish larvae; culture of freshwater fishes is not considered.

EGGS

Sources.—Pelagic fish eggs can be obtained from plankton tows, by catching ripe fish and fertilizing the eggs, and by induction of spawning of laboratory brood stock.

Let eggs taken in plankton tows stand in quart bottles for 0.5 h, then remove plankton from bottom of jar and add fresh sea water (a second decanting may be required). Jars are stored on their sides in an insulated ice box with a refrigerant for 24 h or longer with the temperature kept within spawning range.

Virtually all marine clupeoid fishes (Blaxter and Hunter, 1982) and probably most other pelagic marine fishes spawn at night, hence running ripe fish are more common at night or just before sunset (final egg maturation or hydration occurs just before spawning). After an egg is spawned in sea water its fertility decreases but the maximum time for it to become infertile is highly variable among species, varying from 6 minutes to over 3 hours (Ginzburg, 1972). Sperm in sea water may remain fertile for days (Ginzburg, 1972) although fertility periods as short as 30 seconds have been observed (Haydock, 1971). Owing to the great variation in the time eggs and sperm remain fertile it is preferable that sperm and eggs be mixed immediately after they are obtained.

Storage of gametes may be helpful since mature males and females are not always available simultaneously and crosses between subpopulations may be desired. It is well known that sperm can be stored for extended periods (10 or more hours) if kept cool and maintained in the concentrated form and not activated by sea water (Ginzburg, 1972; Erdahl and Graham, 1980). Fertilization of *Clupea harengus* eggs may be obtained after 6–7 days dry storage at 4° C but a high hatching rate is expected only after periods less than 36 h (Blaxter and Holliday, 1963). It is now possible to extend the life of fish sperm for much longer periods using cryopreservation techniques (−196° C) (Erdahl and Graham, 1980). Various cryoprotective agents have been used to freeze sperm of marine fishes including glycerol (Blaxter and Holliday, 1963), glucose, NaCl, Ringer's solution and fish serum (Hara et al., 1982).

The stress of capture causes female *Katsuwonus pelamis* to ovulate and spawn within 24 h after capture but eggs are often not viable (Kaya et al., 1982). Maturing marine fish in the laboratory and spawning them by hormone injections has become routine in recent years and is preferable to stress techniques. Examples include *Engraulis mordax* (Leong, 1971), *Scomber japonicus* (Leong, 1977), *Chanos chanos* (Liao et al., 1979), *Bairdiella icistia* (Haydock, 1971), *Paralichthys dentatus* and *Pseudopleuronectes americanus* (Smigielski, 1975a, b) and others (see review of Lam, 1982). Induction of spawning in the laboratory may require an open sea water system, large holding tanks (e.g., ~3 m dia. or larger), temperature and light control.

Handling and stocking.—To count eggs without damaging them we recommend a polished wide bore (~3 mm) pipette; count 30–50 late stage eggs at a time in a depression slide under a dissection microscope, and wash eggs off the slide by immersion of the entire slide in sea water. Counting eggs is critical because higher mortalities and slower growth result from excess stocking densities (Houde, 1975 and 1977). As a rule stocking densities in rearing tanks of 8 eggs/l or less seems preferable and most rearing successes have occurred when stocking did not exceed 20 eggs/l (Houde, 1975). Similarly, the mortality of *Mugil cephalus* larvae seems to remain constant (2–3% loss/day) at stocking densities of 1–30 larvae/l (Kraul, 1983).

APPARATUS

Containers and lighting.—Larvae appear to grow faster and show fewer signs of starvation when reared in large containers (100 l) rather than in smaller ones (10 l) (Theilacker, 1980b). Optimum container size doubtless varies with species but 40 l containers are probably the minimum size that should be used and I prefer 100–400 l containers. We use cylindrical black fiberglass containers although excellent results are obtained using ordinary rectangular glass aquaria (Houde, 1975).

It is traditional to provide a daily cycle of illumination to

larvae in rearing containers although constant illumination is occasionally used. Typically fluorescent lamps are used which provide 2,000–3,000 lux at the water surface (Houde, 1978; Hunter, 1976). Night light levels vary; we provide no light at night whereas Houde (1978) provides a dim light of 40–90 lux at night, which is substantially above the visual threshold for feeding for larval *E. mordax* (6 mm larvae 50% feeding threshold = 6 lux, and 10–15 mm larvae 50% threshold = 0.6 lux, Bagarinao and Hunter, 1983). Clearly, longer periods for visual feeding will probably enhance growth if food is limited. Rearing at high light intensities such as natural sunlight may greatly increase production of algae and zooplankton in the culture tank and thereby increase larval survival (Kraul, 1983). On the other hand, solar UV radiation is clearly lethal to younger larvae (Hunter et al., 1982) and use of deep tanks, or shaded or covered tanks (screen cloth, acrylic plastic, glass or mylar film) is recommended for the first 1–2 weeks of larval life if tanks are to be exposed to solar radiation.

Water quality.—Closed, non-circulating systems are typically used to rear marine fish larvae at least during the younger stages, because in an open system planktonic larvae and their foods are easily lost. Older (nektonic) larvae are able to resist the current and to consume a daily ration in a short period so a partially open system can be used. We fill our rearing containers with UV treated sea water that is passed through three, in line, cartridge filters (5, 3 and 1 μm pore).[1] Although not a common practice in small scale rearing work, the addition to rearing tanks of antibiotics (sodium penicillin G at 50 i.u./ml plus streptomycin sulphate at 0.05 g/ml) slightly improved survival of *Pleuronectes platessa* eggs through hatching, but surprisingly this single treatment greatly improved survival of larvae through metamorphosis (Shelbourne, 1975).

Use of a closed system requires attention to water quality, a problem which may be intensified at higher rearing temperatures. In the most complete study of water quality in rearing tanks for marine fish larvae, Brownell (1980a, b) considered seven variables (pH, dissolved oxygen, carbon dioxide, ammonia, nitrite and nitrate), but only high pH, low dissolved oxygen and un-ionized ammonia had effects at levels likely to be encountered in rearing tanks. First feeding incidence declined by 50% in all species he studied when dissolved oxygen concentrations were between 4 and 4.75 mg/l (49–58% saturation). Dissolved oxygen in our rearing containers usually is not saturated after planktonic foods are added, and typically it is about 80% saturation even with aeration. Clearly water quality is improved by aeration and frequent water changes and tank cleaning. Werner and Blaxter (1980) exchanged 20% of the water in *Clupea harengus* cultures (9° C) 3 times per week but at high temperatures greater replacement rates are required. For example Houde (1977) replaced 20% of the tank sea water on alternate days while culturing *Anchoa mitchilli* and *Achirus lineatus* at 26–28° C. Frequent tank cleaning is important as heavy mortalities may result from toxins produced by debris on the container bottom (Kraul, 1983). Aeration, unless very gentle, can cause heavy mortalities among delicate eggs and newly hatched larvae. In fact, Shelbourne (1964) recommends no aeration for *Pleuronectes platessa* larvae. I recommend very gentle aeration but not until a week or so beyond the first feeding stage.

The mortality of cultured fish larvae often increases during the period of initial swim bladder inflation in physoclistous fishes (Doroshev et al., 1981; Kuhlmann et al., 1981) and this could be related to water quality. Symptoms include delay or complete failure of inflation or excessive inflation; in either case normal swimming patterns are disrupted and death frequently results. The causes of abnormal inflation are not clear; prevention of larvae from reaching the water surface prevented excess inflation in *M. cephalus* larvae (Nash et al., 1977), whereas the same treatment in *Atractoscion nobilis* larvae had no effect. In *A. nobilis* excess inflation was associated with abnormal development of gas secretory tissue suggesting a more complex etiology (SWFC, unpubl. data). Failure to inflate the swim bladder is a common problem in *Morone saxatilus* culture and turbulent aeration may reduce the incidence of this disease (Doroshev and Cornacchia, 1979) but it now appears that reduction in salinity from 17 ppt to 4 ppt has a much greater effect in reducing the incidence of swim bladder malfunction (S. Doroshev and J. Merritt, U. Cal. Davis, pers. comm.).

Food

The most critical aspect of rearing marine larvae is management of their food. Food must be the correct density, size, nutritionally adequate and must remain suspended in the water column which usually requires the use of living pelagic organisms.

Food size.—Typical pelagic fish larvae are 2.5–4.0 mm when they begin feeding and acceptable prey are 20–150 μm in breadth (Houde and Taniguchi, 1979). Some large larvae, e.g., larval *C. harengus* (Blaxter, 1965), *Pleuronectes platessa* (Riley, 1966) or small larvae with large mouths, e.g., *Merluccius productus* (Sumida and Moser, 1980), can begin feeding on prey 300 μm or larger in breadth. The optimal food size increases as larvae grow (Hunter, 1981), so any culture technique should provide a steadily increasing range of food sizes, because if the food is too small growth slows and mortality occurs (Hunter, 1981). Food size requirements can be expressed in terms of the ratio of prey width to mouth width. The 50% threshold for feeding on a prey of a particular width occurs when this ratio is about 0.75, although occasionally larvae consume prey as wide as the width of their mouth (ratio = 1) (Hunter, 1981). At the onset of first feeding a small prey of about ¼ the mouth width seems to be preferable as capture success is low at this time but within a few days larvae are able to consume food of about ½ the mouth width.

Wild zooplankton.—Wild zooplankton, primarily the naupliar and copepodite stages of marine copepods but also mollusc veligers, tintinnids, cladocera, and appendicularia larvae, are the natural foods of most marine fish larvae and probably also the best source of food for rearing a larval taxonomic series. Wild zooplankton provide a wide range of sizes and types and are probably nutritionally superior to cultured rotifers and *Artemia* nauplii (Kuhlmann et al., 1981). Collection of wild zooplankton may require less effort than production of cultured food except for brine shrimp nauplii (see below). Zooplankton is collected in nets of about 50 μm, and is graded by size in the laboratory using various nylon nets (Houde, 1977, 1978). This eliminates the larger zooplankton which larvae would be unable

[1] Aqua-Pure model AP10, AMF Cuno Division, Inc., Meriden, Connecticut USA.

to consume and which may be larval predators. Fish larvae, particularly yolk-sac stages, are vulnerable to various carnivorous copepods, amphipods, euphausiids and chaetognaths (Hunter, 1981).

Cultured foods.—Two cultured foods, the rotifer *Brachionus plicatilis*, and nauplii of the brine shrimp, *Artemia*, should be considered as potential foods for rearing marine fish larvae as many fish larvae can be reared on a combination of these two foods. These two foods may also be used as a supplement to diets of wild plankton. Groups of fishes that have been reared to metamorphosis on a combination of *Brachionus* and *Artemia* or on *Artemia* alone include *C. harengus*, species of serranids, scombrids, atherinids, various flatfishes, sciaenids, and saganids (May, 1970; May et al., 1974; and unpubl. SWFC data). *Artemia* nauplii are recommended only for larvae with differentiated guts as they are quite resistant to digestion whereas copepods are not (Rosenthal, 1969).

Methods for culturing rotifers using algae are given by Theilacker and McMaster (1971); culture methods employing formulated artificial diets or freeze dried algae (Gatesoupe and Robin, 1981; Gatesoupe and Luquet, 1981) and ones using brewers yeast also exist. Many of the essential facts given in these original papers will not be repeated here but I will point out a few practical points regarding rotifer culture using algae. Suitable algae species for rotifer culture include *Dunaliella, Nannochloris, Tetraselmis*, and *Chlorella* which may be grown using standard culture media (Guillard, 1975) or using liquid commercial plant fertilizers (dosage for fertilizer containing 8% total nitrogen = 0.1 ml of fertilizer/l; dosage among brands is adjusted depending on total N content). We prefer commercial plant fertilizers that have an organic base such as liquid fish fertilizers and avoid those that have soil penetrants. A daily doubling rate can be expected in healthy rotifer cultures, and cultures can be maintained for weeks or even months by adding fresh algae or nutrients and sea water, although single batch harvesting after about 2 weeks gives more dependable results. Rotifers are harvested using gravity flow through a nylon filter (20–40 μm mesh) as pumps may kill rotifers.

Production of *Artemia* nauplii is simple since all that is needed is to hatch the cysts ("*Artemia* eggs"). Cysts from a variety of strains of *Artemia* are commercially available. The strains differ considerably in average naupliar size (423–775 μm length), in pesticide content (DDT, PCB, and chlordane) and in certain fatty acids (Klein-MacPhee et al., 1982). These authors show that very low survival (15%) of *P. americanus* larvae occurred when they were fed San Pablo Bay (San Francisco) nauplii whereas survival of larvae fed other strains varied from 60–80%. Beck et al. (1980) gave similar results for *Menidia menidia* larvae. Of all the strains tested in these papers the Australian and Brazilian strains seem the most suitable for rearing larvae and the San Pablo Bay (USA) the least.[2]

Artemia hatcheries vary from a jar to complex automated systems. The J. D. Riley *Artemia* hatching box has been used with slight modification in many laboratories for over 20 years. It is a sea water filled box separated in half by a sliding partition; *Artemia* cysts are added to one side (1 g/l) and they hatch 1–2 days later depending on the temperature selected (23–30° C). The tank is then illuminated, the partition raised slightly off the bottom, and the nauplii, attracted by the light, swim beneath the partition leaving behind the hatching debris and unhatched cysts (Shelbourne, 1964). A semiautomatic version of this system is described by Nash (1973), and various other improvements in aeration, illumination, temperature, and other factors have increased yields to 10^6 nauplii per 4.8 g of cysts (San Francisco Bay Brand) (Dye, 1980). In recent years decapsulation of *Artemia* cysts using hypochlorite bleach has become popular because it increases yields, increases the dry weight of the nauplius (Bruggeman et al., 1980) and eliminates contamination of larval fish rearing tanks with unhatched cysts.

It should also be noted that freshly hatched *Artemia* nauplii are clearly more nutritious than older starving individuals and consequently new batches should be frequently produced. In general, prey with full stomachs are probably nutritionally preferable to ones with empty stomachs. Similarly, more *Dicentrarchus labrax* larvae seem to survive when rotifers are nutritionally enhanced by 30 min immersion in a solution containing vitamins and soluble proteins (Gatesoupe and Luquet, 1981).

Mass culture of marine copepods is difficult and laborious and therefore not recommended when a taxonomic series is the sole objective. Nevertheless, culture of marine copepods may be the only way some fish larvae can be reared if wild zooplankton is not readily available and larvae die when fed *Artemia* nauplii (rarely are more than a single strain of *Artemia* tested, however). Harpacticoid copepods (*Tigriopus* sp., *Tisbe* sp., and *Euterpina* sp.) are the most frequently used copepods because of ease of culture; for culture techniques see Kahan et al. (1982) and Hunter (1976). *Euterpina* may be preferable to *Tigriopus* or *Tisbe* because the nauplii and copepodites of *Euterpina* are pelagic and therefore available to the larvae whereas nauplii and copepodites of *Tigriopus* and *Tisbe* tend to remain on surfaces and are therefore less available (Kraul, 1983). See Nassogne (1970) and Zurlini et al. (1978) for laboratory culture of *Euterpina*.

Food density.—The optimal food density for fish larvae depends upon the size of the food organism and size or age of the larvae. Densities of 1–3 organisms/ml have been routinely used for larvae fed wild zooplankton (largely copepod nauplii) during the first 1–2 weeks of feeding (Houde and Taniguchi, 1979). The same density range is used when cultured *Artemia* nauplii are the food. A higher density range (10–20/ml) is used for cultured *B. plicatilis* which are about 1/10 of the weight of an *Artemia* nauplius (Theilacker and McMaster, 1971). A very small food particle, the dinoflagellate *Gymnodinium splendens* (40 μm dia), is used for the first 2 days of feeding in northern anchovy larvae (Lasker et al., 1970; Hunter, 1976) at a high density of about 100/ml. In very active species such as *S. japonicus* or the siganid *Siganus canaliculatus* high food densities can cause heavy mortality because of overfeeding since most larval fishes seem to lack a satiation mechanism (May et al., 1974; Hunter, 1981). Overfeeding seems to occur only when such easily captured prey as *Artemia* nauplii are used as food.

Piscivorous fish larvae.—Piscivorous fish larvae such as the scombroids, *Sphyraena* and others pose special problems in culture. Fish larvae are an ideal food for such larvae; in fact, our only success in rearing *Katsuwonus pelamis* larvae to metamorphosis was probably related to an abundant supply of yolk-

[2] Exotic *Artemia* cysts are available from: Artemia Inc., P.O. Box 2891, Castro Valley, California 94546 USA and Biomarine Research, 4643 W. Rosecrans, Hawthorne, California 90250 USA.

sac fish larvae as food. Zooplankton is the initial food until piscivorous feeding habits develop (Houde, 1972b; Mayo, 1973; Hunter and Kimbrell, 1980). Piscivorous larvae manipulate their larval prey and consequently are less dependent on mouth size when consuming larval fish. Sibling cannibalism is common under rearing conditions in such fishes. Increasing the food density may increase survival as may elevating the temperature, thereby accelerating growth through the most cannibalistic sizes; at least in scombroids sibling cannibalism declines at metamorphosis (Mayo, 1973; Hunter and Kimbrell, 1980). Sorting by size and isolating the larger larvae is probably the only certain method for controlling losses due to cannibalism, however.

Phytoplankton

Phytoplankton blooms are often maintained in larval culture tanks to reduce the detrimental effects of metabolic by-products which accumulate in static rearing tanks (Houde, 1974) and to provide food for larval food organisms. In many cases dense blooms of phytoplankton enhance larval growth and survival and I recommend the practice but the mechanism is obscure. The phytoplankters used are various, easily grown, small species such as *Chlorella, Anacystis, Nannochloris, Tetraselmis, Dunaliella, Isochrysis, Phaeodactylum* and others.[3] They are maintained at high densities (10,000 or more cells/ml) in the rearing tanks. At high cell densities larvae ingest these small phytoplankters, perhaps inadvertently (Moffatt, 1981) but they appear not to be able to exist on them as a sole food source (Houde, 1974; Scura and Jerde, 1977). They may supplement the food ration either directly or indirectly through the ingestion of prey having guts full of algal cells (Moffatt, 1981). Evidence now exists that enhancement of growth and survival of larval *Scophthalmus maximus* by blooms of *Isochrysis* and *Phaeodactylum* is due to the inclusion in the diet of certain polyunsaturated fatty acids not occurring in the normal laboratory rotifer diet (Scott and Middleton, 1979). It is interesting in this regard that *Dunaliella* which lacks the fatty acids did not enhance *S. maximus* larval growth or survival.

Effects of Culture

Extrapolation from cultured larvae to natural populations must be done with caution because culture may affect the morphology, behavior and biochemistry of larvae (Blaxter, 1976). The morphological characteristics most susceptible to modification in tanks are those partially controlled by environmental conditions such as vertebrae and fin ray counts. Reared larvae also may be more heavily pigmented than sea caught specimens (Watson, 1982). This appears to be related to the expanded nature of the melanophores, not to added numbers of pigment cells. In addition, pigmentation events may occur at smaller sizes in reared material (S. Richardson, Gulf Coast Research Laboratory, Ocean Springs, Mississippi, pers. comm.). Laboratory reared larvae are often heavier and have deeper bodies than their wild counterparts, making some morphometric measurements on laboratory specimens useless (Blaxter, 1975). The differences in preservation and handling between laboratory and sea-caught larvae also make direct size-specific comparisons difficult. Shrinkage in length may vary greatly depending on the duration larvae remain in plankton nets and shrinkage differences between reared and wild specimens can be misinterpreted as morphological differences (Theilacker, 1980a).

National Marine Fisheries Service, Southwest Fisheries Center, P.O. Box 271, La Jolla, California 92038.

[3] For a nominal fee starter cultures of marine phytoplankton can be obtained from R. R. L. Guillard, Bigelow Laboratory for Ocean Sciences, McKown Point, West Boothbay Harbor, Maine 04575 USA; culture methods are discussed by Guillard (1975).