Hatchery techniques for specific diversification in Mediterranean finfish larviculture

Divanach P., Kentouri M.

Recent advances in Mediterranean aquaculture finfish species diversification

Zaragoza : CIHEAM
Cahiers Options Méditerranéennes; n. 47

2000
pages 75-87

Hatchery techniques for specific diversification in Mediterranean finfish larviculture

P. Divanach* and M. Kentouri*,**
*Department of Aquaculture, Institute of marine Biology of Crete, Heraklion, Crete, Greece
**Department of Biology, University of Crete, Heraklion, Crete, Greece

SUMMARY – After a very early research stage in the 70's, and a predevelopment stage in the 80's, the Mediterranean finfish mariculture (mainly sea bass sea bream) has boomed in the 90's, reaching about 70,000 t of marketable size fish and 300 million fry in 1997. The main reason for this success was the progress and diversification in hatchery techniques. All based on the use of live prey, these techniques are divided into several categories according to larval density, (intensive, mesocosms and extensive), the quality of the rearing medium (clear water, green water, pseudo green water) and some other criteria. Although perfectible, these techniques are actually quite satisfactory for species diversification in many fields of larviculture, but their choice has to be well documented. In this article, the authors review the conditions of this challenge.

Key words: Mediterranean aquaculture, larval rearing, hatchery techniques.

RESUME – *"Techniques en écloseries pour la diversification des espèces en culture larvaire de poissons méditerranéens". Après une étape très précoce de recherche dans les années 1970, et un stade de pré-développement pendant les années 1980, la mariculture de poissons méditerranéens (principalement loup et dorade) a connu une très forte expansion dans les années 1990, atteignant environ 70 000 t de poissons de taille commercialisable et 300 millions d'alevins en 1997. Les principales raisons de cette réussite étaient le progrès et la diversification des techniques en écloseries. Toutes basées sur l'utilisation de proies vivantes, ces techniques sont divisées en plusieurs catégories selon la densité larvaire (intensive, mésocosme et extensive), la qualité du milieu d'élevage (eau claire, eau verte, et eau pseudo-verte) et certains autres critères. Bien que perfectibles, ces techniques sont en réalité tout à fait satisfaisantes pour la diversification des espèces dans plusieurs domaines de la larviculture, mais leur choix doit être bien documenté. Dans cet article, les auteurs font une révision des conditions de ce défi.*

Mots-clés : *Aquaculture méditerranéenne, élevage larvaire, techniques en écloseries.*

Introduction

Hatcheries are the infrastructures for mass production of fry, the limiting factor of further ongrowing operations. From the beginning of Mediterranean finfish mariculture in the 70's, their technical mastery is a requisite for industrial development and an important R&D concern. The recent orientation towards species diversification re-emphasizes their role.

At the onset of the new millennium, there is not yet any technique which allows the rearing of marine finfish larvae with inert food from the beginning of heterotrophic life. But the range of available hatchery techniques with live prey is quite diverse. The main classifications are based on the rearing density (intensive, mesocosms, extensive) and the quality of water (clear water, green water, pseudo green water). Each of them subdivided into variants according to the origin of water (open sea or littoral well), the type of hydroid circuit (open or recycled, depurated or not), the type of feeding or enrichment of prey (with phytoplankton, artificial food, emulsion rich in poly-unsaturated fatty acids (PUFA), proteins, vitamins) and the type of innovations aimed at simulating the functions of the green water (with phytoplakcton conserved in heterotrophy, refrigerated, frozen, or with simple organic products).

But the definitions, limits and conditions for use of these categories remain so vague that misunderstandings and errors during applications are frequent. They often lead to bad biological and economical results with new species (mortalities, decalibration, deformities, etc.). The objective of this article is to review the main characteristics of these techniques and to propose some elements for their better utilisation with new (i.e. little known) species.

Techniques of larviculture

These belong to three main categories (intensive, mesocosms, extensive) which range between three orders of magnitude of larval density from 0.1-1 larva/litre (extensive technique) to 150-200 larvae/litre (hyperintensive technique) (Fig. 1). This classification, which defines the reputation of productivity and efficiency, also integrates very different structural and operational characteristics (Table 1) (Divanach, 1985).

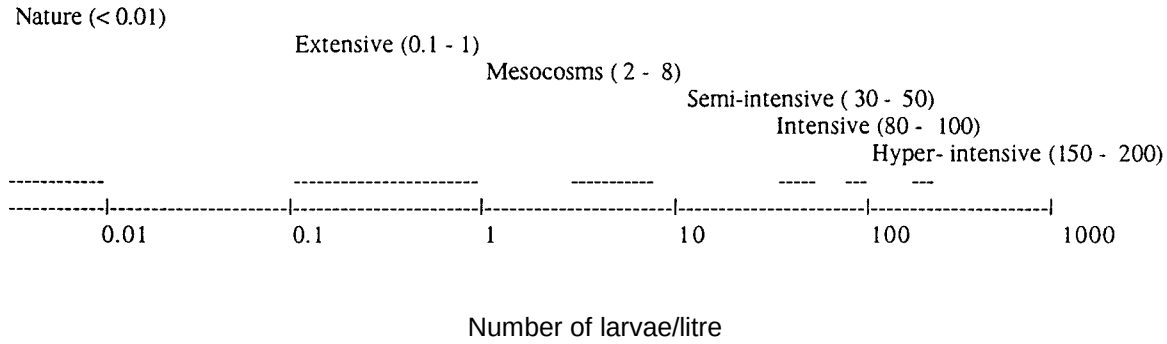

Fig. 1. Classification of technologies according to larval density.

Table 1. Main differences between hatchery techniques

Parameters	Techniques		
	Extensive	Mesocosm	Intensive[†]
Rearing enclosures	Ponds or bags	Tanks or bags	Tanks
Localisation	Outdoor	In door[††]	Indoor
Rearing volume (m^3)	>100	30-100	<20
Rearing density (ind/l)	0.1-1	2-8	30-200
Food chain	Endogenous	Mixed	Exogenous
Infrastructures	Light	Medium	Sophisticated
Environment	Natural	Mixed	Controlled
Autonomy and autarky	High	Medium	Low to nil
Dependence on man and technique	Light	Medium	High to very high
Need for specific biological knowledge	Light	Medium	High to very high
Validity for new species	Very high	High	Medium to low

[†]Integrate semi-intensive, intensive and hyper-intensive techniques.
[††]Sometimes outdoor (with bags) or semi-outdoor.

Intensive techniques

These are expensive, sophisticated indoor-rearing techniques in which production depends totally on man and technology. They are characterized by high larval densities in small, well shaped, (often cylindroconical) tanks, under strict specific hydraulic, thermic, light and feed conditions. They present three variants (semi-intensive = 30-50 larvae/litre; intensive = 80-100 larvae/litre; hyper-intensive = 150-200 larvae/litre) whose complexity increases with the larval density (Lagos 1989; Kentouri *et al.*, 1993; Dhert *et al.*, 1998).

The photoperiod, intensity and spectrum of light are artificial, often changing with the age of larvae. The temperature and the quality of water, as well as the hydrodynamism, are fully controlled. The geometry and organisation of the tank (colour of the walls and bottom, position and orientation of inlet and outlet of water, of the lamps, feeders, skimmer, aeration) are very precise in order to allow both

the homogenous behaviour of all the larval population and good ergonomical conditions for easy management of tanks (feeding, flushing out of sedimented wastes, etc.). The cleaning of the water surface (by skimmer) of any oily film, a requisite for good swimbladder inflation and absence of related lordoses, is absolutely synchronised with the period and conditions of air gulping by larvae. The food is totally exogenous and restricted to a diet of just *Brachionus plicatilis* and *Artemia*, two live prey that do not occur in the natural environment of the cultured larvae and need special enrichment [proteins, highly unsaturated fatty acids (HUFA), vitamins] in order to match the metabolical requirements of larvae. Mass production of these prey in special parallel infrastructures is totally synchronised with larviculture. The adequate frequency, intensity and duration of prey distribution, which are requisite for high survival, high calibration and absence of cannibalism in larvae, are made by experienced staff or by adequate automation. The accelerated increase of needs for water, food and space with the increase of biomass necessitates permanent readequation of supply and the rearing process has to be segmented (with change of tank, sorting and manipulation of fry) between one and two months.

With these techniques, all parameters are potentially limiting and the requisition for success is a high level of specific biological knowledge and practical know how. The dependence on man (monitoring and know how) and technology (electricity, pumping, spare pieces, automatisms, alarms, etc.) is important. Specialisation of tasks and duplication of teams are often needed to provide a good 24 hour/day survey. Nights, week ends and holidays, during which staff is restricted and less specialised, are often problematic periods. In case of default, even provisional, the autarky of the system is limited and its survival very short (less than one hour for oxygen).

When the technical supply does not totally match the biological demand, larvae have no other solution than to adapt or to die. When adapting, they sometimes become deformed (non marketable) or handicapped (poor growth performances). All the small inadequacies occurring during larviculture (even a priori successful) have consequences. Decalibration, deformations, anomalies of coloration and behaviour, cannibalism, selection of low growers, abnormal fry sex ratio (selective male production with sea bass) are the most frequent problems observed.

Thus, only few marine species (*Dicentrarchus labrax, Sparus aurata, Diplodus sargus, Solea solea* and *Scophtalmus maximus*) are actually so well known as to be reared successfully on an industrial scale with this method.

The sophistication and cost of both infrastructures and operations (economical break point up to 1.5-2 million fry/year), make these techniques impracticable to small producers and a risk for new species.

Extensive techniques

These are cheap ecological techniques based on the productivity of artificial oligospecific pelagic marine ecosystems in which larvae are the upper level of the food chain and the supposedly unique beneficiaries of the matter fluxes. They derive from techniques used for centuries in continental pisciculture. Rearing volumes are filled, inoculated with phytoplankton and zooplankton, populated with fish eggs or larvae and incubated under such natural conditions as to create a food chain providing the necessary and sufficient flux of matter towards larvae until they reach a size compatible with crop. Two main variants are used: (i) a short cycle (1-2 months) providing ready-to-wean post larvae for intensive aquaculture; and (ii) a longer one (2-6 months) providing metamorphosed fry (or juveniles) for repopulation or extensive aquaculture (Divanach and Kentouri, 1982; Houde and Lubbers, 1986).

Larviculture is performed at low densities (0.1-1/litre) in large (hundreds to thousands of m^3), deep (2-5 m) outdoor volumes (generally ponds, sometimes plastic bags, rarely fine mesh cages or tanks) which have been prepared to provide adequate feeding to larvae larvae (Divanach and Kentouri, 1990). Volumes are so big that modifications of environment are difficult. Cultures are exposed without protection to all climatic fluctuations and adapt to natural environment, with regional, seasonal and geographic specificities. Long (>18 hours) photoperiod and temperate (15-21°C) temperatures are generally optimal, but some species (such as sea bass) may also be reared in temperate winter conditions. Only minor actions (artificial lighting by night, partial shadowing in summer, use of transparent plastic film cover in inter-season) can provide a cheap increase of productivity. When climatic conditions are too rough (winter in northern regions and summer in southern ones), production is impossible and the ponds are used for other purposes (Divanach and Kentouri, 1984). The rearing

medium is sea water taken from coastal sea, lagoon or littoral well. Water is used directly after rough filtration on 250-350 µm mesh (for elimination of potential predators/competitors), never thermoregulated, eventually fertilized, sometimes chlorinated (when containing undesirable food chains) and then reinoculated with new food chains. No special action for surface film elimination is needed as wind blows it naturally. Once tanks are filled, renewal of the medium is generally low or nil.

Feeding is exclusively provided by an endogenous bloom of zooplankton which itself develops on endogenous primary productions (Paulsen *et al.*, 1985). Exogenous feeding is (normally) not provided, the system being autarchic from the beginning of trophic life until the crop. Two main food chains are used (cf. Sections on Natural bloom method and Green water method). The first one, which consists in a bloom of wild diversified marine (or lagoon) plankton (Harris, 1982; Nixon *et al.*, 1984; Divanach and Kentouri, 1990; Kuuppo *et al.*, 1994), is of high nutritional/vitamin value and does not need any enrichment. It is valid for very difficult species. Furthermore, as it often contains meroplanktonic forms, it provides benthos for post larvae of necto-benthic fish when they metamorphose, thus allowing production of old fry. The second, which consists in the bloom of the bicomponent domesticated food chain *Chlorella* (or *Nanochloropsis*)-*Brachionus plicatilis*, is a variant of the so-called green water technique. It is often easier to manage, but less lasting and has a lower performance. The two food chains are partially incompatible as they need different levels of eutrophy (Pitta *et al.*, 1993, 1996, 1998).

With these techniques, the success of the production is based on the intensity and the longevity of the food chain (Gamble, 1985; Frank and Leggett, 1986). When producers make the mistake of over reaching the biotic capacity of the ecosystem, autarky is limited to few days (or weeks), causing problems of growth, decalibration and cannibalism in larvae with subsequent bad production (Divanach, 1985; Oiestad, 1985). The conditions for success are knowledge of the ecosystem and respect for the ecosystem laws of productivity. The youth and oligospecific organisation of the food chain, conditions for priority development of pelagic species versus bentho, protection against larval competitors and predators, adequation to regional geography and seasonal climatic conditions and respect for initial density and age of larvae are the main factors of productivity. The related know-how is the basis of the technology.

The role of man is less important than in intensive approach. The problems of management during weekends, night and holidays are unknown. The main human operations are the preparation of the pond, the initialisation of the food chain, the inoculation of eggs or larvae and the final fishing of fry. The quality of the initial organisation is primordial because reorientation/reactivation of the food chain during culture is generally non-effective (too late for larval demand). On the opposite the role of nature is preponderant. If climatic conditions are bad, man (generally) cannot avoid them.

Even when not well mastered, the diversity of the environmental and feeding conditions offered by these techniques allows such a multitude of biological/ethological choices that larvae may find suitable preferences and are not forced to adapt stringently (Kentouri and Divanach, 1982a,b, 1983b, 1986a,b; Kentouri *et al.*, 1983). They always express important biological rhythms. Patchiness and differential repartition correlated with light, currents or zooplankton concentrations, as well as daily vertical and horizontal migrations are observed. As a consequence, the probability of success is much higher than in the intensive approach. About 20 species are already reared using this technique, of which several have very small eggs (*Lithoghnathus mormyryus, Puntazzo puntazzo*), normally considered more difficult than the others. Also, the quality of fry is very good. The problems of swimbladder inflation, skeletal deformities, anomalies of coloration and behaviour are almost unknown (Koumoundouros *et al.*, 1995; Divanach *et al.*, 1996). With sea bass, the anomaly of sex ratio is not observed. Growth performances of fry during ongrowing is much better than that of those from intensive culture.

Although low, the rearing densities with these techniques are from hundreds to thousands of times higher than natural ones. Fry recruitment, when successful in such volumes, may be very important reaching to several hundreds of thousands (if not millions) of individuals. The challenge for industrial application is either to increase the productivity of medium volumes (cf. Section on Mesocosm techniques: mesocosm strategy) (Drenner *et al.*, 1990) or to increase the mastery of production in very big volumes (megacosms) of several thousands (if not millions) of cubic meters (Gamble *et al.*, 1985).

Mesocosm techniques

These are indoor or semi-outdoor hatchery techniques. They are intermediate between the semi-

intensive and extensive one, and so can be considered as semi-extensive techniques of mass production. They are relatively recent in their actual form and were defined in the early 90's after study of the intrinsic qualities and faults of their original models (Grice and Reeves 1982; Bever *et al.*, 1985; Lalli, 1990).

They present two variants and four subvariants according to the origin and the quality of the food chain (Fig. 2). In the first, of extensive philosophy, the food chain is basically endogenous and complemented with exogenous input when presenting symptoms of overgrazing. In the second, of intensive philosophy, the food is basically exogenous but presents a capacity of endogenous reproduction due to both the low density (i.e. the low grazing impact) of larvae, and the presence of phytoplankton in the environment. The four subvariants characterised by the methods for improvement of environment and/or food chain are described in Section on Associated methods for improvement of rearing medium and/or food chain.

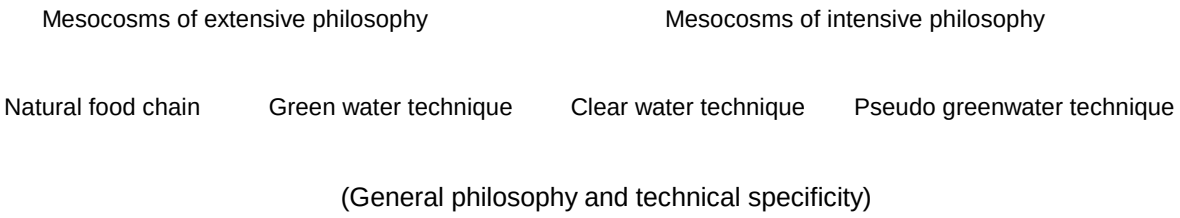

Fig. 2. Organisation of mesocosm technologies.

Mesocosms are high-performing techniques of larviculture which have the advantages of both intensive and extensive techniques without their inconveniences. Larvae are reared at relatively low densities (2-8/litre) in relatively large (30-100 m^3), deep (1.5-2.5 m), well shaped tanks (sometimes bags) located in well organised installations. These ensure quality of ergonomy, security of rearing, high number of fry produced per tank (50,000 to 300,000 according to species) and high human productivity (>2 millions fry/man/year). Environmental conditions are both natural and artificial, providing a variety of possible preferendum, while avoiding any climatic, seasonal or geographic limitations and allowing optimisation of operational costs. Long photoperiod (>18 hours) and temperate (15-21°C) temperatures are preferred by a majority of species. The cleaning (by skimming) of oily film from water surface is needed for maximal (100%) swimbladder inflation, as nature cannot provide it alone. Feeding, from both endogenous and exogenous origin, ensures a good matching of larval energy requirements, while avoiding any possibility of vitamin deficiency and any risk of over grazing of the food chain. This partial autarky, a type of passive automatic feeding, is very important for the quality and cost effectiveness of production by night and during weekends.

The biological performances of known species (*Dicentrarchus labrax, Sparus aurata, Diplodus sargus*), are often better than that obtained with intensive or extensive techniques. Survival after weaning generally ranges between 40 and 90% of eggs (mean about 60%); total deformities between 1-5% (of which opercular deformities less than 2-3% for sea bream); swimbladder inflation close to 100%; growth between 15 and 20 mg at one month with very high calibration; homogenous behaviour without any cannibalism (i.e. no need for sorting) during the first 40-50 days; weaning totally achieved at one month. With turbot, almost all the population present normal wild coloration.

The validity of mesocosms for new species is similar to that of extensive techniques. They have been used successfully for fry production of 25 fish species and 5 hybrids, of which 17 have been referenced at international level (Table 2) and the rest reported in grey literature. Of this total, a majority was produced only with mesocosms of extensive philosophy and natural food chain (*Anchoa mitchilii, Gadus morhua, Clupea harengus, Pleuronectes platessa, Hippoglossus hippoglossus, Solea solea, Scophtalmus maximus, Brevortia* sp.). But others, such as *Dicentrarchus labrax, Sparus aurata, Puntazzo puntazzo, Diplodus sargus, Diplodus vulgaris, Diplodus annularis, Lithognathus mormyrus, Pagrus pagrus, Dentex dentex,* were also produced with the green water method or in mesocosms of intensive philosophy with the pseudo green water method. With many of these species, survival was higher than 20% despite very low previous specific knowledge and know how.

Table 2. Main references of marine fish species reared in mesocosms[†]

Species	Country[††]	Type[†††]	Reference
Anchoa mitchilii	US	A	Cowan and Houde, 1990
Gadus morhua	No.	A	Ellertsen *et al.*, 1981; Oiestad, 1984; Oiestad *et al.*, 1985; Van der Meeren, 1991
Clupea harengus	No.	A	Oiestad and Moksness, 1981; Moksness and Oiestad, 1987; Wespestad and Moksness, 1989
Pleuronectes platessa	Dk.	A	Danielsen *et al.*, 1981
Hippoglossus hippoglossus	No.	A	Oiestad and Berg, 1989; Rabben *et al.*, 1986
Solea solea	Dk.	A	Berg *et al.*, 1985
Scophtalmus maximus	No.	A	Berg *et al.*, 1985; Paulsen and Andersen, 1989
Brevortia sp.	US	A	Keller *et al.*, 1990
Dicentrarchus labrax	Fr.	A	Hussenot *et al.*, 1991
	Fr.	A C	Nehr, 1996
Sparus aurata	Fr.	A	Divanach, 1985; Kentouri, 1985; Divanach and Kentouri, 1983a; Pitta, 1996; Giannakourou, 1995
		A B	Dhert *et al.*, 1998
		A B C	Kentouri *et al.*, 1995a
Lithognathus mormyrus	Fr.	A	Divanach and Kentouri, 1983b,c; Kentouri and Divanach, 1982a, 1983a,b
Puntazzo puntazzo	Fr., Gr.	A C	Divanach and Kentouri, 1982; Divanach *et al.*, 1993
Diplodus sargus	Fr., Gr.	A C	Kentouri and Divanach, 1986b
Pagrus pagrus	Gr.	B	Ben Khemis, 1997; Kentouri *et al.*, 1995b,c
Pagrus major	Jp.	A B	
Dentex dentex	Gr.	A C	Kentouri *et al.*, 1995; Koumoundouros *et al.*, 1999
Diplodus annularis	Gr.	A C	Divanach *et al.*, 1995; Kentouri *et al.*, 1995b

[†]In the grey literature, positive results of larviculture have been obtained with "mesocosm type" methods with *Sciena umbrina, Pagrus major, Uranoscopus scaber, Scorpaena porcus, Pagellus acarne, Pagellus erythrinus, Boops boops, Mugil cephalus*.
[††]US: USA; No.: Norway; Dk.: Denmark; Fr.: France; Gr.: Greece; Jp.: Japan.
[†††]Type of initial mesocosm technology: A = Extensive or semi extensive with natural food chain; B = Semi extensive with green water method; C = Semi extensive with pseudo green water method.

Associated methods for improvement of rearing medium and/or food chain

Each of the previous techniques is generally characterised by some complementary method for environment and/or food chain management (Table 3). The main associations are described hereafter and their characteristics summarised in Table 4.

Table 3. Main associations of methods and techniques in Mediterranean finfish hatcheries

Technique	Method					
	Natural	Green	Pseudo green	Neogreen	Clear	No name
Extensive	+	+	–	–	–	–
Mesocosms	+	+	+	–	–	+
Semi intensive	–	+	+	–	+	–
Intensive	–	–	+	+	+	+
Hyper intensive	–	–	+	+	+	+

Table 4. Main differences between methods according to rearing medium/food chain

Parameters	Method					
	Natural	Green	Pseudo green	Neogreen	Clear	No name
Microalgal back ground	Yes	Yes	Yes	Yes	No	No
Type of phytoplankton	Live	Live	Live	Preserved	–	–
Origin of phytoplankton†	Endo	Endo	Exo	Exo	–	–
Quality of environment††	Evol	Evol	Stable	Evol	Stable	(?)

†Endo = endogenous; Exo = exogenous.
††Evol = evoluting.

Natural bloom method (or natural food chain)

This method, used in extensive technique and in mesocosms of extensive philosophy, is based on the bloom of a pelagic food chain of wild origin which associates three characteristics: (i) a slightly greenish quality of the environment with diversified successive populations of phytoplankton, mainly diatoma (*Skeletonema*, *Chaetoceros*), picoplankton, flagellates, dinoflagellates; (ii) an associated characteristic succession of zooplanktonic populations (nude and loricated ciliates, rotifers *Synchaeta*, copepods *Acartia, Temora, Oithona*; and (iii) then a benthic population induced by the fixation of meroplanktonic larvae inherent in the water (Divanach and Kentouri, 1990; Pitta *et al.*, 1993, 1996, 1998; Kentouri *et al.*, 1995a).

Tanks are filled with natural sea water filtered through a coarse plankton mesh (250-350 µm) eliminating potential predators/competitors while allowing the small wild plankton to be used as inoculum. At the same time, the water is fertilized with 0.4-0.5 g/l N and 0.1-0.15 g/l P, sometimes slightly inoculated (in very oligotrophic regions) with *Chlorella* (15,000-20,000 cells/ml) or *Tetraselmis suecica* (500-1000 cells/ml) and incubated under temperate conditions (16-20°C) and high permanent illumination (25-30 W neon cool white/m^2). Sea water renewal ranges between 0.5-2%/day. After filling, 8 to 15 days are needed for optimal development of the zooplanktonic segment edible by fish larvae (tintinids 1-5/ml between 5 and 10 days; *Synchaeta* 0.2-2/ml between 8 and 15 days; nauplii and metanauplii of copepods between 15 and 25 days). The total duration of the high performing pelagic zooplanktonic food chain (tintinids, *Synchaeta*, copepods) is about 20-25 days, long enough for larvae to reach a size compatible with Artemia feeding (even often with direct artificial food).

This food chain is considered the best for larviculture of all very new and difficult species (Kentouri and Divanach, 1986b), although it may sometimes create problems when contaminated with too many undesirable species (bloom of medusae, medusoids of fixed hydraria, trochophores of epibenthic annelids). Furthermore, its value for production of larval biomass is often less than that of the green water method (cf. Section on Green water method). Any attempts made to increase its productivity (through increased fertilisation, or eutrophy, or temperature), as well as the trials of mixed culture with the *Chlorella-Brachionus* food chain (green water method), have generally been non-effective (extinction or replacement).

Green water method

This method, used in extensive techniques and in mesocosms of extensive philosophy is derived from that used in the 60's in Japan for larviculture of *Pagrus major*. It consists of the development of an endogenous bicomponent bloom of *Chlorella* (or *Nanochloropsis*) and *Brachionus plicatilis* in a tank which is then inoculated with fish larvae (Ben Khemis 1997; Ben Khemis and Divanach, 1999).

Clean empty tanks are filled with axenic sea water, fertilized with 1 to 2 g N and P/m^3, contaminated with microalgae (0.05-0.1 million cells/ml) and rotifers (0.1-0.2 individuals/ml) from parallel intensive culture and then incubated at 18-22°C under permanent illumination (natural by day, artificial cool white neon 20-30 W/m^2 by night). There is no water renewal. Previous chlorination (1 p.p.m. NaoCl during 12 hours with strong aeration) and then neutralisation with thiosulfate of the remaining chloride is sometimes needed for elimination of undesirable wild food chains prior to

inoculation. Four to eight days, according to temperature, are needed for optimal development of the phytoplanktonic (0.5-2 millions cells/ml) and zooplanktonic (1-5 rotifers/ml) segments. The introduction of fish eggs or prelarvae in the tank is made at such a time when larvae enter trophic life and the rotifer bloom is up to 2/ml.

The behaviour and the life time of this eutrophic food chain is highly correlated to larval density (Divanach and Kentouri, 1990; Kentouri *et al.*, 1995a). When too low (less than 1-2 larvae/l), the rotifers quickly bloom, overgraze the phytoplankton, and then die in an exhausted yellowish medium. The correlated brutal change of the quality of the environment (one night) is often responsible for a lethal drop of pH and oxygen in larvae. On the other hand, when too high (>7-10 larvae/l), the rotifer population is quickly overgrazed by larvae and the remaining phytoplankton blooms to more than 3-5 million cells/ml, leading to both a superficial hyperoxygenation and anoxia at the bottom of the tank (due to the low light penetration). The consequences for larvae are either gas bubble disease or hypoxia, both responsible for high mortalities. When populated (i.e. grazed) correctly by larvae, the food chain is stable and often lasts more than 25 days, more than enough for them to adapt to instar 1 of *Artemia* and ready for weaning. But its quality generally decreases after 15-20 days, generating bacterial disorders (increase of gram negative populations) and nutritional deficiencies (increase of carbohydrates and lowering of proteins) in the prey. Therefore, a new strategy of management is needed (opening of the circuit and use of either the pseudo green or the clear water method).

Pseudo-green water method

This method, used in intensive technique and in mesocosm of intensive philosophy, is based on larviculture in an environment of rather stable composition containing phytoplankton (*Chlorella* or *Nanochloropsis*) and rotifers (*Brachionus plicatilis*) from exogenous origin (parallel intensive cultures), readjusted daily in relation to larval demand.

Three variants of the method are possible: in the first (pure) one, the tank is filled with clear sea water and populated with fish eggs or prelarvae which develop in an almost axenic water. The phytoplankton and rotifers are added only when larvae begin the heterotrophic life. In the second and in the third, a natural food chain (cf. Section on Natural bloom method) or a *Chlorella-Brachionus* one (cf. Section on Green water method) are initiated prior to addition of eggs or prelarvae. Part of the autotrophic phase occurs in a very populated environment which may sometimes perturb prelarvae. The pseudo-green water method, applied as soon as larvae begin trophic life, provides a double endogenous exogenous feeding from the beginning.

The illumination provided by both artificial and natural sources is permanent and maintained between 100-200 lux (night) and 2000-10,000 lux (day). Sea water renewal ranges between 2 (beginning) to 20 (end) %/day. The phytoplankton from exponential phase culture is added 1 to 5 times daily in a range of 0.1-0.2 million cells/ml. The rotifer density is adjusted to 2-5 individuals per ml by addition of enriched (HUFA, vitamins, proteins) individuals. The addition of phytoplankton is generally maintained until larvae reach a size allowing feeding with instar 2 of *Artemia*. Then the culture is managed as in the clear water method.

This method performs well for both quality and quantity of production (Scott and Baynes, 1978; Moffatt, 1981; Naas *et al.*, 1991; Van der Meeren, 1991; Reitan *et al.*, 1993; Tamaru *et al.*, 1994; Kennedy *et al.*, 1998).

Clear water method

This method, used in intensive techniques and in mesocosms of intensive philosophy, is based on larviculture in an environment of clear sea water without any phytoplankton background (except the possible light natural one inherited with the pumped sea water, or with the rotifers when feeding). Rearing is always associated with a high rate of water renewal (>10%/hour) which avoids any endogenous microalgal development. The hydraulic circuit can be direct or recycled after depuration.

This method, which is not adequate for long survival of rotifers in the tank (lack of feeding background), is generally only used with species which quickly accept instar 1 of *Artemia* (as with sea

bass). Or it is associated with systems of automatic feeding which provide, through frequent distributions, a constant quality and quantity of prey. But the matching of larval requirements is always more difficult than with other methods. Remaining rotifers quickly sediment on the bottom where they can only survive for a short time, filtering bacteria and detritus. Then they die, contributing to high bacterial contamination (mainly gram negative) of the sediment.

With some species, such as *Sparus aurata,* which are not resistant to water renewal at the beginning of trophic life (unknown reason), this method does not give good results. Furthermore, it often induces bacterial pathologies (thiorhodobacteria, pasteurellosis) in larvae, possibly for the precited reason of feeding inadequacy and rotifer mortalities (Kennedy *et al.*, 1998).

Other methods

Some other methods aiming to simulate the role and functions of the green water background during larviculture while avoiding the problems of *in situ* production, are beginning to be used at a research level.

In the neo-green method, the phytoplankton added to the tanks is previously conserved in heterotrophy at a very high density (some grams/litre) in bottles at ambient temperature, or as a live paste in a refrigerator at 4°C. A variant of this method makes use of frozen microalgae (–18°C). In other methods, which have not yet been named, the products added to the tanks are not microalgae but phytoplankton extracts or organic products. They have not yet been well evaluated.

Conclusion

The lack of appropriate tools for larviculture of new species has, for a long time, delayed diversification in marine finfish production. The actual panoply of hatchery techniques and associated methods is such that any range of ecological and biological conditions, i.e. any gradient of complexity and of difficulty of adaptation for larvae, can be simulated. Even with little known species, blanks with high biological performances (survival, growth, conformity with wild standard, etc.) are possible.

The extensive method is a natural, empiric but easy and effective way of screening the first needs of unknown species and to provide sufficient biological material for further studies. The challenge for its competitive industrialisation is the mastery of very large volumes (megacosms), which will open the markets of repopulation and sea ranching, the basis of future sustainable fisheries.

The semi-extensive mesocosm technology is an intermediate, more productive and more analytical approach to begin metabolic studies by modification of the biochemical or environmental supply in the most difficult segments of the rearing procedure. Up to now, it is a very competitive technology for species diversification and probably the future tool for production of difficult marine species such as Seriola, Tuna, groupers, etc.

The intensive method is the final approach, and one in which all parameters have to be mastered for success and which represents the top level of knowledge and productivity. However, the long time needed for research and then the transfer of knowledge, often make it non-competitive compared to mesocosms during the predevelopment stage when market supply is low and benefits potentially high.

References

Ben Khemis, I. (1997). *Elevages larvaires de poissons méditerranéens: Optimisation de la production en mésocosme et diversification des espèces*. Thèse Européenne Université de Droit, d'Economie et des Sciences d'Aix-Marseille III.

Ben Khemis, I. and Divanach, P. (1999). Elevages larvaires de poissons marins: Evolution de la technologie semi-extensive en mésocosmes orientés et perspectives de développement. *J. Stat. Biol. Salambo* (in press).

Berg, L., Baaroy, V., Danielsen, D.S., Van der Meeren, T., Naas, K.E., Senstad, K. and Oeistad, V. (1985). *Production of juvenile flatfish species in different sized mesocosms*. Comm. Meet. Int. Counc. Explor. Sea C.M., ICES/F: 65.

Bever, J.E., Christensen, E.B., Christensen, V., Kiorboe, T., Munk, P., Paulsen, H. and Richardson, K. (1985). Summary of Danish enclosure experiments. Comm. Meet. Int. Counc. Explor. Sea C.M., ICES, Mini Symp. No. 7 (mimeo).

Cowan, J.H. Jr. and Houde, E.D. (1990). Growth and survival of bay anchovy *Anchoa mitchilli* larvae in mesocosm enclosures. *Mar. Ecol. Progr. Ser.*, 68: 47-57.

Danielsen, D.S., Moksness, E. and Oiestad, V. (1981). Duoculture of plaice (*Pleuronectes platessa* L.) and lobster (*Homarus gammarus* L.) fry in two concrete enclosures based on natural production. *Rapp. P.-v. Réun. Cons. Int. Explor. Mer*, 178: 511-513.

Dhert, P., Divanach, P., Kentouri, M. and Sorgeloos, P. (1998). Rearing techniques for difficult marine fish larvae. *World Aquaculture*, March: 48-55.

Divanach, P. (1985). *Contribution à la connaissance de la biologie et de l'élevage de 6 sparidés méditerranéens:* Sparus aurata, Diplodus sargus, Diplodus vulgaris, Diplodus annularis, Lithognathus mormyruus, Puntazzo puntazzo. Thèse de Doctorat ès Science, Université des Sciences et Techniques du Languedoc (Montpellier II).

Divanach, P., Boglione, C., Menu, B., Koumoundouros, G., Kentouri, M. and Cataudella, S. (1996). Abnormalities in finfish mariculture: An overview of the problem, causes and solutions. In: *Seabass and Seabream Culture: Problems and Prospects*. Verona, pp. 45-66.

Divanach, P. and Kentouri, M. (1982). Utilisation des techniques extensives pour la production à grande échelle d'alevins de sar *Puntazzo puntazzo*. *C. R. Acad. Sci. Paris*, 294(3): 1017-1019.

Divanach, P. and Kentouri, M. (1983a). Influence des conditions trophiques initiales sur la résorption des réserves lipidiques, la croissance et la survie des larves de daurade *Sparus aurata* en elevage extensif. *Aquaculture*, 35: 43-55.

Divanach, P. and Kentouri, M. (1983b). Développement embryonnaire et larvaire du marbré *Lithognathus mormyrus* en élevage extensif. *Cybium*, 7(4): 89-103.

Divanach, P. and Kentouri, M. (1983c). Données préliminaires sur la technique de production, la croissance et la survie des larves de marbré *Lithognathus mormyrus*. *Aquaculture*, 31: 245-256.

Divanach, P. and Kentouri, M. (1984). Sur les possibilités de production de juvéniles de possons marins par la filière extensive dans le Languedoc. Influence de l'époque et des conditions climatiques. In: *L'Aquaculture du Loup et des Sparidés*, Barnabé, G. and Billard, R. (eds). INRA Publ., Paris, pp. 175-184.

Divanach, P. and Kentouri, M. (1990). Larval rearing in extensive conditions. In: *Aquaculture*, Vol. 2, Barnabé, G. (ed.). Ellis Horwood Publ., pp. 820-832.

Divanach, P., Kentouri, M. and Sterioti, A. (1993). The use of mesocosmes in the mass production of *Puntazzo puntazzo* fry. In: *Management of fresh water and estuarine ecosystems, 6th Pan-hellenic Congress of Ichthyologists*, Xanthi, 4-6 June, p. 10 (in Greek).

Divanach, P., Kentouri, M. and Sterioti, A. (1995). Use of mesocosm method for mass production of Diplodus annularis fry. In: *8th Panhellenic Congress of Ichthyiologists*, Bolos, 22-24 September 1995.

Drenner, R.W., Smith, J.D., Mummert, J.R. and Lancaster, H.F. (1990). Responses of a eutrophic pond community to separate and combined effects of N:P supply and planktivorous fish: A mesocosm experiment. *Hydrobiologia*, 208: 161-167.

Ellertsen, B., Moksness, E., Solemdal, P., Tilseth, S., Westgard, T. and Oiestad, V. (1981). Growth and survival of cod larvae in an enclosure. Experiments and a mathematical model. *Rapp. P.-v. Réun. Cons. Int. Explor. Mer*, 178: 45-57.

Frank, K.T. and Leggett, W.C. (1986). Effect of prey abundance and size on the growth and survival of larval fish: An experimental study employing large volume enclosures. *Mar. Ecol.-Prog. Ser.*, 34: 11-22.

Gamble, J.C. (1985). *More space for the sparsely distributed: An evaluation of the use of large enclosed experimental systems in fish larval research*. ICES, Council Meeting Copenhagen (Denmark), 14 pp.

Gamble, J.C., Maclachlan, P. and Seaton, D.D. (1985). Comparative growth and development of autumn and spring spawned Atlantic herring larvae reared in large enclosed ecosystems. *Mar. Ecol.-Prog. Ser.*, 26: 19-33.

Giannakourou, A. (1995). *Réseaux trophiques planctoniques utilisables pour l'élevage larvaire de la daurade*. PhD Thesis, Université de Sciences et Techniques du Languedoc, Montpellier II.

Grice, G.D. and Reeves, M.R. (eds) (1982). *Marine Mesocosms, Biological and Chemical Research in Experimental Ecosystems*. Springer-Verlag, New York.

Harris, R. (1982). Comparison of the feeding behaviour of *Calanus* and *Pseudocalanus* in two experimentally manipulated enclosed ecosystems. *J. Mar. Biol. Assn. UK*, 62: 71-91.

Houde, E.D. and Lubbers, L. (1986). Survival and growth of striped bass, *Morone saxatilis*, and

Morone hybrid larvae: Laboratory and pond enclosure experiments. *Fish. Bull.*, 84(4): 905-914.

Hussenot, J., Lemoine, O. and Lagardere, F. (1991). L'élévage larvaire extensif du Bar (*Dicentrarchus labrax*) et de la Daurade royale (*Sparus aurata*) en milieu naturel stimulé par fertilisation dans les marais salés de la côte Atlantique, France. *ICES Mar. Sci. Symp.*, 192: 127-136.

Keller, A.A., Doering, P.H., Kelly, S.P. and Sullivan, B.K. (1990). Growth of juvenile Atlatic menhaden, *Brevoortia tyrannus* (Pisces: Clupeidae) in MERL mesocosms: Effects of eutrophication. *Limnol. Oceanogr.*, 35(1): 109-122.

Kennedy, S.B., Tucker, J.W., Neidig, C.L., Vermeer, G.K., Cooper, V.R., Jarrell, J.L. and Sennett, D.G. (1998). Bacterial management strategies for stock enhancement of warmwater marine fish: A case study with common snook *Centropomus undecimalis. Bull. Mar. Sci.*, 62(2): 573-588.

Kentouri, M. (1985). *Comportement larvaire de 4 Sparides méditerranéens en élevage:* Sparus aurata, Diplodus sargus, Lithognathus mormyrus, Puntazzo puntazzo *(Poissons téléostéens)*. Thèse de Doctorat ès Sciences, Université de Sciences et Techniques du Languedoc, Montpellier.

Kentouri, M. and Divanach, P. (1982a). Comportement et régime alimentaire des larves de marbré *Lithognathus mormyrus* élevées dans des conditions de choix trophique polyspécifique et pluridimensionnel. *C. R. Acad. Sci. Paris,* 194(3): 859-861.

Kentouri, M. and Divanach P. (1982b). Différences et similitudes dans la génèse des comportements locomoteurs et trophiques des stades prelarvaires de *Sparus aurata*, *Diplodus vulgaris*, et *Diplodus sargus*. *Aquaculture*, 27: 355-376.

Kentouri, M. and Divanach, P. (1983a). Contribution à la connaissance du comportement et de la biologie des larves de marbré *Lithognathus mormyrus* (Sparidae) en élevage. *Ann. Zootech.*, 32(2): 135-152.

Kentouri, M. and Divanach, P. (1983b). Sur l'utilisation des critères comportementaux pour déterminer l'état de santé et l'évolution probable des élevages de poissons marins. 1. Cas des prélarves et des larves de *Diplodus sargus*, *Sparus aurata*, *Puntazzo puntazzo*, *Lithognathus mormyrus*. In: Bases Biologiques de l'Aquaculture. *IFREMER*, *Actes de Colloques*, 1: 525-538.

Kentouri, M. and Divanach, P. (1986a). Spectres alimentaires des larves de Sparides en condition controlées. Sélectivite spécifique de la daurade *Sparus aurata*. *Oceanol. Acta*, 9(3): 343-348.

Kentouri, M. and Divanach, P. (1986b). Sur l'importance des ciliés pélagiques dans l'alimentation des stades larvaires de poissons. *Année Biologique*, 25(4): 307-316.

Kentouri, M., Divanach, P., Papandroulakis, N. and Stefanakis, S. (1993). Biological performance of intensively reared sea bream (*Sparus aurata*) larvae in a pilot closed system. In: Proceeding of the World Aquaculture '93. *EAS Special Publication*, 19: 396.

Kentouri, M., Divanach, P. and Paris, J. (1983). Approche du comportement trophique des larves de *Diplodus sargus*, *Sparus aurata*, *Puntazzo puntazzo* et *Lithognathus mormyrus*. In: *L'Aquaculture du Bar et des Sparides*, Barnabé, G. and Billard, R. (eds). INRA Publ., Paris, 139-149.

Kentouri, M., Divanach, P., Sterioti, A., Papapetrou, M., Maingot, E., Barnabe, G., Yannakourou, A., Ben Khemis, I., Connes, R., Diaz, J.P. and Guyot de la Hardrouyere, E. (1995a). *A comparative study of cost effectiveness with growth performances of sea bream* Sparus aurata *larvae reared under two different climatic conditions in France and Greece using three types of Mesocosms.* Report of the AQ. 2. 429 FAR project.

Kentouri, M., Papandroulakis, N. and Divanach, P. (1995b). Specific diversification in Greek finfish mariculture. In: Aspects Economiques de la Production Aquacole, IAM Montpellier, 17-19 May 1995. *Cah. Options Méditerr.*, 14: 129-136.

Kentouri, M., Pavlidis, M., Papandroulakis, N. and Divanach, P. (1995c). *Culture of the red porgy,* Pagrus pagrus*, in Crete. Present knowledge, problems and perspectives*. TECAM, Nicosia, Cyprus, 14-17 June 1995.

Koumoundouros, G., Divanach, P. and Kentouri, M. (1999). Ontogeny and allometric plasticity of *Dentex dentex* (Osteichtyes: Sparidae) in rearing conditions. *Mar. Biol.*, 135: 561-572.

Koumoundouros, G., Gagliardi, F., Divanach, P., Stefanakis, S. and Kentouri, M. (1995). Osteological study of the origin and development of the abnormal caudal fin in gilthead sea bream *Sparus aurata* fry. In: Quality in Aquaculture*,* Svennevig, N. and Krogdahl, A. (eds). *EAS Special Publication*, 23: 16-18.

Kuuppo, L.P., Autio, R., Hallfors, S., Kuosa, H., Kuparinen, J. and Pajuniemi, R. (1994). Trophic interactions and carbon flow between picoplankton and protozoa in pelagic enclosures manipulated with nutrients and a top predator. *Mar. Ecol.-Prog. Ser.*, 107: 89-102.

Lagos, P.J. (1989). An integrated marine fish farm in Cephalonia, Greece. II. An analysis and evaluation of economic performance. In: *Aquaculture – A Biotechnology in Progress*, De Pauw, N., Jaspers, E., Ackefors, H. and Wilkins, N. (eds). European Aquaculture Society, Bredene (Belgium), pp. 1175-1181.

Lalli, C.M. (1990). *Enclosed experimentale marine ecosystems: A review and recommendations. Coastal and marine studies*. Springer-Verlag.

Moffatt, N.M. (1981). Survival and growth of northern anchovy larvae on low zooplankton densities as affected by the presence of a Chlorella bloom. *Rapp. P.-v. Réun. Cons. Int. Explor. Mer*, 178: 475-480.

Moksness, E. and Oiestad, V. (1987). Interaction of Norwegian spring-spawning herring larvae (*Clupea harengus*) and Barents Sea capelin larvae (*Mallotus villosus*) in a mesocosm study. *J. Cons. Int. Explor. Mer*, 44: 32-42.

Naas, K.E., Naess, T. and Harboe, T. (1991). Enhanced first feeding of halibut larvae (*Hippoglossus hippoglossus* L.) in green water. *Aquaculture*, 105: 143-156.

Nehr, O. (1996). *Développement d'une méthodologie de production semi intensive d'alevins en mer dans des enceintes à renouvellement semi continu: Application à l'élevage du loup* Dicentrarchus labrax. PhD Thesis, Centre d'Océanologie de Marseille, Univ. de la Méditerranée.

Nixon, S.W., Pilson, M.E.Q., Oviatt, C.A., Donaghay, P., Sullivan, B., Seitzinger, S., Rudnick, D. and Frithsen, J. (1984). *Flows of Energy and Material in Marine Ecosystems*, Fasham, M.J.R. (ed.). Plenum Press, New York, pp. 105-135.

Oiestad, V. (1984). Criteria for condition evolved from enclosure experiments with cod larva populations. 1. The propagation of cod *Gadus morhua* L. *Flodevigen Rapportser,* pp. 213-229.

Oiestad, V. (1985). Predation on fish larvae as a regulatory force, illustrated in mesocosm studies with large groups of larvae. *NAFO Sci. Coun. Studies*, 8: 25-32.

Oiestad, V. and Berg, L. (1989). Growth and survival studies of halibut (*Hippoglossus hippoglossus* L.) from hatching to beyond metamorphosis carried out in mesocosms. In: *Aquaculture – A Biotechnology in Progress*, De Prauw, N., Jaspers, E., Ackefors, H. and Wilkins, N. (eds). European Aquaculture Society, Bredene (Belgium), pp. 233-240.

Oiestad, V., Folkvord, A. and Kvenseth, P.G. (1985). *Growth-patterns of Atlantic cod larvae (*Gadus morhua *L.) from first feeding to metamorphosis studied in a mesocosm*. ICES, Council Meeting, Copenhagen, 18 pp.

Oiestad, V. and Moksness, E. (1981). Study of growth and survival of herring larvae (*Clupea harengus* L.) using plastic bag and concrete basin enclosures. *Rapp. P.-v. Réun. Cons. Int. Explor. Mer*, 178: 144-149.

Paulsen, H. and Andersen, N.G. (1989). Extensive rearing of turbot larvae (*Scophthalmus maximus* L.). In: *Aquaculture – A Biotechnology in Progress*, De Prauw, N., Jaspers, E., Ackefors, H. and Wilkins, N. (eds). European Aquaculture Society, Bredene (Belgium), pp. 241-248.

Paulsen, H., Munk, P. and Kiorboe, T. (1985). *Extensive rearing of turbot larvae (*Scophthalmus maximus *L.) on low concentrations of natural plankton*. Comm. Meet. Int. Counc. Explor. Sea C.M., ICES/F: 33/sess. Q.

Pitta, P. (1996). *Dynamique du système planctonique en mésocosmes d'élevage de daurades*. PhD Thesis, Université de Crète.

Pitta, P., Divanach, P. and Kentouri, M. (1993). Nano and microplankton in Iraklion Bay: Comparison of coastal waters and mesocosm enclosure assemblages. Paper presented at *28th European Marine Biology Symposium*, 23-28 September 1993, Hersonissos, Crete (Greece).

Pitta, P., Giannakourou, A., Divanach, P. and Kentouri, M. (1996). Planktonic food web in large enclosures in the eastern Mediterranean. From pico- to microplankton. In: *Eutrophication in Planktonic Ecosystems. Food Web Dynamics and Elemental Cycling, Proceedings of International PELAG Symposium*, 26-30 August 1996, Helsinki, Finland, p. 30.

Pitta, P., Giannakourou, A., Divanach, P. and Kentouri, M. (1998). Planktonic food web in marine mesocosms in the Eastern Mediterranean: Bottom-up or top-down regulation? *Hydrobiologia*, 363: 97-105.

Rabben, H., Nilsen, T.O., Huse, I. and Jelmert, A. (1986). *Production experiment of halibut fry in large enclosed water columns*. ICES, Council Meeting 1986/F: 19.

Reitan, K.I., Rainuzzo, J.R., Oie, G. and Olsen, Y. (1993). Nutritional effects of algal addition in first-feeding of turbot (*Scophthalmus maximus* L.) larvae. *Aquaculture*, 118: 257-275.

Scott, A.P. and Baynes, S.M. (1978). The effect of unicellular algae on survival and growth of turbot larvae (*Scophthalmus maximus* L.). In: *Proc. World Symp. on Finfish Nutrition and Fishfeed Technology*, Vol. 1, Hamburg, 20-23 June 1978. Heenemann Verlagsgesellschaft mbH, Berlin, pp. 423-433.

Tamaru, C.S., Murashige, R. and Lee, C.-S. (1994). The paradox of using background phytoplankton during the larval culture of striped mullet, *Mugil cephalus* L. *Aquaculture*, 119: 167-174.

Van der Meeren, T. (1991). Algae as first food for cod larvae (*Gadus morhua* L.): Filter feeding or ingestion by accident? *J. Fish Biol.*, 39: 225-237.

Wespestad, V.G. and Moksness, E. (1989). Observations on growth and survival during the early life history of Pacific herring *Clupea pallasii* from Bristol Bay, Alaska, in a marine mesocosm. *Fish. Bull., U.S.*, 88: 191-200.

Further reading

Lancaster, H.F. and Drenner, R.W. (1990). Experimental mesocosm study of the separate and interaction effects of phosphorus and mosquitofish (*Gambusia affinis*) on plankton community structure. *Can. J. Fisheries Aquat. Sci.*, 47: 471-479.

Mazumder, A., MCQuen, D.J., Taylor, W.D. and Lean, D.R.S. (1988). Effects of fertilization and planktivorous fish (yellow perch) predation on size distribution of particulate phosphorus and assimilated phosphate: Large enclosure experiments. *Limnol. Oceanogr.*, 33(3): 421-430.

Mazumder, A., Taylor, W.D., MCQueen, D.J. and Lean, D.R.S. (1989). Effects of fertilization and planktivorous fish on epilimnetic phosphorus and phosphorus sedimentation in large enclosures. *Can. J. Fisheries Aquat. Sci.*, 46: 1735-1742.

Mazumder, A., Taylor, W.D., MCQueen, D.J. and Lean, D.R.S. (1990). A comparison of lakes and lake enclosures with contrasting abundances of planktivorous fish. *J. Plankton Res.*, 12(1): 109-124.

Oiestad, V. (1982). Application of enclosures to studies on the early life history of fishes. In: *Marine Mesocosmes, Biological and Chemical Research in Experimental Ecosystems*, Grice, G.D. and Reeve, M.R. (eds). Springer-Verlag, New York, pp. 49-62.

Threlkeld, S.T. and Drenner, R.W. (1987). An experimental mesocosm study of residual and contemporary effects of an omnivorous, filter-feeding, clupeid fish on plankton community structure. *Limnol. Oceanogr.*, 32(6): 1331-1341.