Development of California Halibut, *Paralichthys californicus*, Culture

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ABSTRACT. In contrast to freshwater aquaculture and the culture of anadromous species such as salmon, marine fish culture is in its infancy. The small larval size of many marine species presents significant challenges to culture, however, these highly valuable fish offer considerable promise for aquaculture. A particularly attractive group for marine aquaculture is the flatfish. The California halibut, *Paralichthys californicus*,

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with a range in nature from Washington State south to Baja California Sur, Mexico is one such species.

With the goal of enhancing the fishery for this species, a hatchery program was developed over a decade ago. The hatchery at Redondo Beach, California, maintains a group of adults that routinely spawn throughout most of each year. Further development of routine culture and juvenile growout techniques ultimately aimed at commercial aquaculture was initiated last year with support from the California Sea Grant College Program.

Profitable commercial ventures culturing various flatfish species already exist in other parts of the world, but development of a flatfish culture industry in California confronts unique challenges. Two challenges in particular are the relatively high cost of energy and stringent environmental regulations. To meet these challenges, a culture system built around recirculation technology is being developed that would allow for an energy-efficient industrial-like approach to the culture of California halibut while minimizing environmental impacts.

KEYWORDS. California halibut, *Paralichthys californicus*, aquaculture, larvae, nutrition, recirculation

INTRODUCTION

The California halibut, *Paralichthys californicus*, is a highly sought after flatfish supporting both commercial and sport fisheries along the Pacific coast of the United States and Mexico (Allen 1990; Kramer et al. 2001). Commercial landings in California once reached over 2,000 MT, but have declined since 1920 to present levels of less than 500 MT per annum. The specific cause for the decline is debatable, but a variety of size, gear, and season restrictions have been ineffective in restoring catches to anywhere near historical levels. This suggests some element of habitat limitation early in the species' life cycle. A likely candidate for this environmental bottleneck is the shallow water embayments used as nursery areas by early juveniles (Kramer 1990, 1991). These nursery areas with relatively low levels of predators, abundant food, and warm temperatures support high survival and growth during the first year of age. Kramer et al. (2001) note that the "overall decline in

California halibut landings corresponds to a decline in shallow water habitats in southern California associated with dredging and filling of bays and wetlands." Once juveniles have grown to between 18-22 centimeters in total length, they migrate from these nursery areas to deeper areas of the coast and are ultimately recruited into the fishery (Kramer 1990). Accordingly, building California halibut populations beyond present levels requires either restoration of lost nursery habitat or bypassing this bottleneck by rearing juveniles in captivity to an appropriate size for release into the coastal environment.

Enhancement of fishable populations through stocking of larger juveniles into the environment would be possible if the larger juveniles could be produced cheaply and effectively (Hobbs et al. 1990). This was the goal of a conservation hatchery project for the California halibut established in the 1990s along with one for white seabass through the California Ocean Resources Enhancement and Hatchery Program (Drawbridge and Kent 2001). Through this program, annually spawning broodstock fish were established at the California Halibut Hatchery at Redondo Beach. While the Hatchery has carried out some limited restocking, the effectiveness of this effort has yet to be quantified.

Recently, the value of hatcheries and restocking programs has come into question particularly in conjunction with mitigation efforts for salmon (Levin et al. 2001). Concern for the long-term health of a fish population is intensified when numbers of hatchery fish are relatively large and their genetic makeup arises from a limited number of founders. On the other hand, enhancement may be useful in local areas where identified recruitment bottlenecks can be bypassed by release of appropriately selected stock (Blankenship and Leber 1995; Blaxter 2000). Studies on stock enhancement of Japanese flounder, *Paralichthys olivaceus* showed a 30% recapture rate, and the benefit-cost ratio estimated to be more than 300% in some areas (Masuda and Tsukamoto 1998). In a study of turbot in Danish waters, Stottrup et al. (2002) released 10,000 small juveniles annually over an eight year period with a no evidence of a negative impact on the wild stock. In spite of these few promising experiments, in general, recovery marine fisheries, including flatfish, that have been overfished is an unpredictably and lengthy endeavor (Hutchings 2001).

An alternative for the consumer is to produce market-sized animals through commercial aquaculture. The high value of flatfish along with their unique association with the benthic environment makes flatfish of particular interest to aquaculturists. As juveniles and adults, these fish spend much of their time lying motionless on the bottom, thus food conversion will be particularly advantageous to the culturist. In addition, provision of inputs such as oxygen and removal of metabolic wastes are less of a challenge than for actively swimming species. Commercial culture of the Japanese flounder, *Paralichthys olivaceus*, is well developed in Asia and culture industries focused on various other species are beginning to emerge in Europe along with a few other spots in the world (see Table 1). In the U.S. commercial culture is just beginning, but a decade of research on the Eastern seaboard is nearing fruition with a few commercial farms now producing summer and southern flounder (Luckenbach et al. 2002).

To meet the objective either of restocking with large juveniles or commercial culture of market-sized animals, the husbandry requirements must be defined so that effective culture systems can be designed. The object of this paper is to bring together the existing data on the California halibut and, where appropriate, on related species to provide the biological foundation for the development of aquaculture of this species either for the purpose of culturing juveniles for stock enhancement or

TABLE 1. Aquaculture production of various flatfish species in 1997 (adapted from data from FAO 1999).

Common Name	Species Name	Country	Metric Tons
Japanese flounder	*Paralichthys olivaceus*	Japan	8,500
		Korea	26,000
		Total	35,000
Atlantic halibut	*Hippoglossus hippoglossus*	Iceland	2
		Total	2
Common sole	*Solea vulgaris*	Portugal	7
		Spain	18
		Total	25
Brill	*Scophthalmus rhombus*	Portugal	20
		Total	20
Turbot	*Scophthalmus maxima*	France	980
		Portugal	25
		Spain	1,800
		Total	3,001
Unidentified species		Chile	278
		Russian Fed.	20
		Total	298
All flatfish species		Global total	38,000

for growout of market-sized animals. Specifically, this paper reviews the efforts at the University of California, Davis in conjunction with the California Halibut Hatchery to develop culture techniques appropriate for California, namely intensive recirculation production systems. While the focus of existing research in California is primarily on commercial culture of foodfish either onshore in recirculating systems (Conklin et al. 2002) or offshore in association with oil platforms (Sylvia et al. 2002), techniques can easily be applied to production of juveniles for stocking purposes.

BROODSTOCK

As indicated earlier, the goal of culturing the California halibut is greatly enhanced by the availability of an existing hatchery program. Few details are available on those factors inducing spawning of the halibut (Caddell et al. 1990). In the wild, peak spawning periods are in the winter and spring (Moser and Watson 1990). Temperature seems to be a key factor controlling reproduction, in that females stop producing eggs if the water temperature stays above 20°C for extended periods (J. Rounds, personal communication, Redondo Beach, California). The broodstock fish at the California Halibut Hatchery are maintained in large outdoor tanks. The largest broodstock tank is 150 m^3 and contains 5 adult females and 10 adult males. A smaller broodstock tank is 37 m^3 and contains 4 adult females and 8 adult males. Both tanks are open at the top although covered with shade cloth and are provided with flow-through ocean water. As such, the broodstock fish are essentially exposed to natural conditions with regard to variations in water temperature and photoperiod. There is also an attempt to provide as natural a diet as possible. Broodstock are fed predominantly with either fresh sardines, anchovies, or squid. In an effort to insure no micronutrient deficiencies develop, the fish are also fed on a weekly basis "brownies" made up of a mixture of a commercial salmon diet, a marine grower diet, plus a vitamin pre-mix containing, among others, stabilized vitamin C and thiamine. These ingredients are bound together with gelatin and cut into appropriate-sized cubes.

LARVAL REARING

Depending on seawater temperatures, eggs typically can be collected from March through August from the broodstock maintained at the California Halibut Hatchery. Egg production can be 40 million eggs per year with roughly 50% viability. Spawning typically occurs in the afternoon with the eggs being collected at the end of the day. Eggs are collected at the outflow of either tank in a fine mesh collecting basket. Harvested eggs are concentrated on a 400-500 μ mesh screen and rinsed with filtered seawater. The eggs are then resuspended in a 3-L container and volumetric samples are taken for determining total eggs collected. Fertilized eggs are allowed to separate from the non-viable and unfertilized eggs, which sink to the bottom of the container. The viable eggs (~ 0.7 mm) are stocked in conical incubators (60 L) at 50-100 eggs/L with a gentle flow of seawater or air to avoid excess turbulence. Gadomski and Caddell (1991) found normal development of eggs at 12, 16, and 20°C. Development was arrested at the 32-cell stage at 8°C and abnormal at 24°C.

It has been found that California halibut eggs immediately after fertilization (between 3 and 17 hours post-fertilization), are sensitive to handling stress (Bush et al. 2002). Consequently, the fertilized eggs should be harvested, rinsed, and transferred to an incubator as soon as possible after fertilization. Further manipulation or shipment of the eggs should be delayed until the eggs have reached an advanced stage of development. At 18-20°C, hatching begins around 36-48 hours after spawning. Both fertilized eggs and larvae have been successfully shipped by air and land to the University of California facilities. Developing eggs and or larvae are shipped at a density of approximately 1,000 per L. Shipping is done in plastic bags to which oxygen has been added to provide a head space of about 35% of the closed bag volume. The bags are placed in insulated containers to provide temperature stability during shipment.

Newly-hatched larvae are about 2 mm in length and begin feeding 3 days post-hatch (dph). First feeding does not coincide with the opening of the mouth, which happens 1 to 2 days earlier, but with development of eye pigmentation. Food is provided during the second day to insure food is available when the mouth opens. The larvae are fed first with rotifers, *Brachionus plicatilis*. In initial trials, the rotifers were enriched with Rotimac™ (Bio-Marine, Inc., Hawthorne, California[1]), but later trials have been more successful using preserved algae *Isochrysis* (Reed Mariculture, San Jose, California). While larval growth is retarded with any delay in first feeding the point of no return does not occur until between 6 and 8 dph at 18°C (Gisbert et al. 2003). Gadomski and Caddell (1991) found increasing the culture temperature hastened starvation. At

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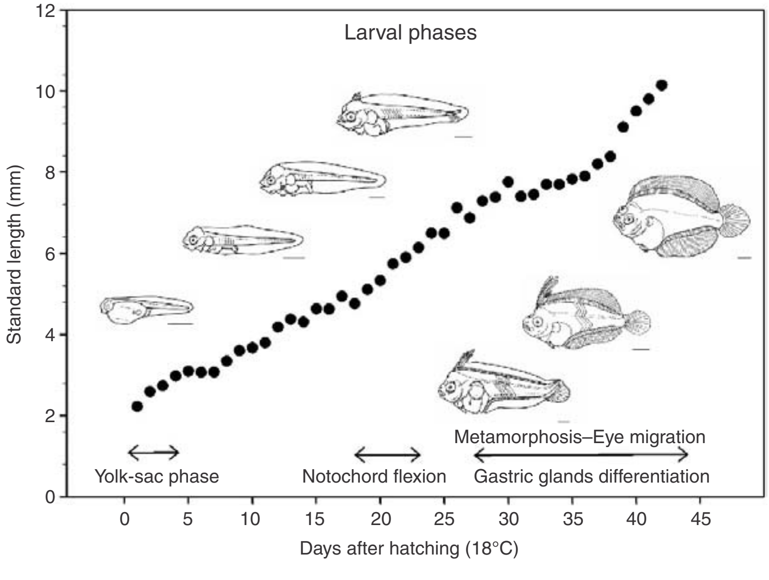
[1] Use of trade or manufacturer's name does not imply endorsement.

18°C, well-fed larvae grow rapidly (Figure 1) and complete metamorphosis by 42 dph (Gisbert et al. 2002). As larvae grow, approximately 17 dph, enriched *Artemia* nauplii are substituted for the rotifers. Weaning to formulated ratios begins around 30 dph. The complete transition to formulated diets from brine shrimp nauplii is accomplished once the fish have undergone metamorphosis and taken up a benthic existence. The time needed for transition to formulated diets is variable depending both on the vitality of the larvae at the time of metamorphosis and the formulated diet used. Good results were obtained with Biokyowa Fry Feed (Kyowa Hakko Kkogyo Co., Tokyo, Japan) and especially with Nippai Ambrose Feed (Dainichi Corp., Uwajima City, Japan).

JUVENILE REARING

Benthic juveniles are cultured at the University of California Davis site in a recirculated culture system (Piedrahita et al. 2002). The basic system has a total volume of approximately 3.5 m^3 and consists of four

FIGURE 1. Growth in standard length of California halibut from hatching to the completion of metamorphosis (modified from Gisbert et al. 2002).



raceways (2.4 m long, 22 cm wide, with a water depth up to 22 cm), a reservoir, a suspended media biological water treatment unit, a constant head tank, a pump plus an alarm and backup system (Figure 2). Temperature is maintained at 21°C with a combination heater/chiller unit. Various other rearing units can be added into the system for carrying out specific experiments. Seawater for the system is trucked from the coast and after chlorination/dechlorination stored in an outdoor tank until needed. The salinity is maintained between 28 and 32 ppt by the addition of dechlorinated tap water. A similar system for larger juveniles is located at the Bodega Marine Laboratory, Bodega Bay, California. The primary differences for the Bodega system are that circular tanks are used instead of raceways and because of the availability of piped seawater at the laboratory, the system is typically operated in a semi-recirculated manner with a constant input of raw seawater. However, when there are interruptions in the laboratory's seawater supply, the Bodega Marine Laboratory system can also be operated in the closed recirculation mode.

Growth rates of early juveniles reared on Biokyowa diet at 21°C were attractive and compared well with other flatfish (Table 2). Studies to define optimum husbandry conditions, such as temperature and salinity tolerance, are in progress. The recent work by Madon (2002) suggests that early juveniles can tolerate a greater range of salinities and temperature than older juveniles which are normally found in coastal environments rather than in embayments. Several studies suggest the

FIGURE 2. Schematic diagram of UC, Davis rearing systems (adapted from Piedrahita et al. 2002).

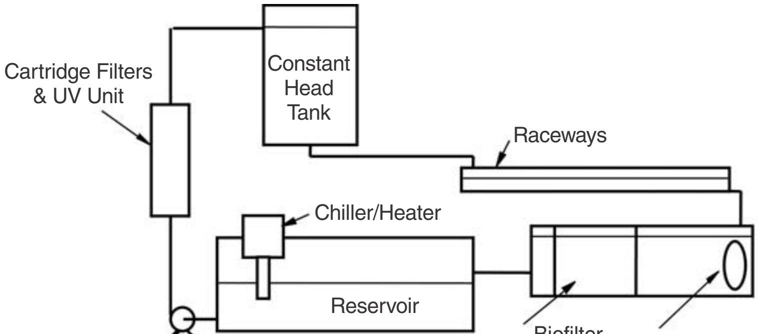


TABLE 2. Specific growth rate (SGR, %/day) of flatfish aquaculture candidates (adapted from Sampaio et al. 2001; unpublished California halibut data from University of California, Davis).

Species	Size range (g)	SGR
Japanese flounder, *Paralichthys olivaceus*	88-120	1.51
Brazilian flounder, *Paralichthys orbignyanus*	3-136	1.06
California halibut, *Paralichthys californicus*	0.2-4.2	1.16
Chilean flounder, *Paralichthys adspersus*	44-157	0.52
European flounder, *Pleuronectes flesus*	18-59	0.60
Smooth flounder, *Liopsetta putnami*	8-35	0.62
Turbot, *Scophtalmus maximus*	5-148	1.56
Winter flounder, *Paralichthys lethostigma*	9-97	0.72
Winter flounder, *Pseudopleuronectes americanus*	8-44	0.71

upper temperature for all stages will be 28°C (Gadomski and Caddell 1991; Madon 2002). As with other flatfish (King et al. 1997; Fairchild and Howell 2001), density of juveniles can be significantly greater than 100% coverage of the bottom. While good results were achieved on Biokyowa diet as well as Nippai diets, the recent outbreak of bovine spongiform encephalopathy (BSE) in Japan has made importation of Japanese diets difficult, accordingly, all juveniles and sub-adults are now reared on EWOS feeds (EWOS Canada Ltd., Surrey, Canada). Nutritional needs as well as optimum levels of protein and energy have yet to be determined. Presently, experiments are being carried out to define digestibility of various feedstuffs and energy requirements of the California halibut juveniles so that experiments to define these nutritional parameters can be carried out. Of particular importance is the definition of optimum protein levels so as to reduce the amount of waste nitrogen that must be dealt with by the biological filter component of recirculation systems.

DEVELOPMENT OF COMMERCIAL PROTOTYPE SYSTEMS

Potential aquaculture endeavors in California will have to fit within the economic and regulatory climate of the state, including the relatively high cost of energy and the recent uncertainty associated with its availability, as well as stringent environmental regulations. It is for these reasons that our program to advance culture techniques appropriate for California halibut is focused on the development of a recirculation growout system. This would allow for establishment of industrial-like culture facilities at a variety of sites within the state. Recirculation will allow for the retention of heat energy used to elevate water temperatures so as to maximize growth rates as well as minimizing any impact on the environment. In keeping with this overall goal, future aims of the project are to bring together the information presently being gathered on optimum husbandry parameters, diet, as well as engineering aspects relating to feeding and the removal of wastes so that prototype systems can be designed. These prototype systems will be built at the Bodega Marine Laboratory. While initial system design may require significant input of fresh seawater, the eventual goal is to reduce this to less than 5% of system volume per day. Once this target is reached, it is thought that serious consideration can be given to moving from prototype systems to commercial reality.

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