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Annotated Bibliography

ADVANCES IN DIAGNOSIS AND MANAGEMENT OF AMYLOODINIOSIS IN INTENSIVE FISH CULTURE

Blaylock, R

Anderson, R. M. and May, R. M. 1979. Population biology of infectious diseases: Part I. Nature 280:361-367.

This is the foundational paper that established the modern concept of epidemiological modelling. The paper argues for viewing the host population as a dynamic variable so as to unite the medical and ecological view of diseases. As such, the host is viewed as a habitat for the parasite, the degree to which the parasite diminishes the reproductive capability of the host is considered, the importance of acquired immunity is considered, and the degree to which host life cycle structure determines transmission from one host to the next is incorporated. The result is a framework for building a simple model that can be tested with real world data.

Lotz, J. M. and Soto, M. A. 2002. Model of white spot syndrome virus (WSSV) epidemics in *Litopenaeus vannamei*. Dis. Aquat. Org. 50:199-209.

The authors present a methodology for experimentally illuminating the population dynamics of a significant pathogen of farmed shrimp. The paper first develops a generalized diagrammatic view of the various states of the host population and the manner in which the virus "flows" through or connects the states of the host population. Connections are then transformed into a series of mathematical equations and a laboratory challenge system is used to provide experimental estimates of the rates of those connections. The authors evaluate the relative importance of the various connections in the progress of an epidemic and conclude that a threshold density of susceptible shrimp exists below which an epidemic will not occur. Increases in transmission efficiency lower the threshold value while most other factors increase it. Epidemic models suggest testable hypotheses that may contribute to the development of control strategies for WSSV outbreaks.

Masson, I., Blaylock, R.B., and Lotz, J.M. 2011. Susceptibility and tolerance of spotted seatrout, *Cynoscion nebulosus*, and Red Snapper, *Lutjanus campechanus*, to experimental infections with *Amyloodinium ocellatum*. J. Parasitol. 97:577-585.

The authors adapt the Lotz and Soto approach to a stage-structured model of an ectoparasite. Components are the life-history stages of the parasite and connections are the transitions between stages. Two species' ability to harbor the dinoflagellate are compared. Experimental estimates of the transitions are achieved. There were no differences in infection rates or lethal doses between the red snapper and spotted seatrout, but trophonts grew larger and remained attached longer to red snapper. The trophont lethal load was higher in spotted seatrout.

A proposed model reflected the preliminary experimental findings and provides a foundation for building an epidemiological model.

Mori, Y. and Notomi, T., 2009. Loop-mediated isothermal amplification (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases. J. Infect. Chemother. 15:62-69.

The authors, one of whom published the original paper on LAMP, review the technology and advantages of the technique compared to other techniques such as PCR and other DNA amplification methods. The advantages include the possibility of using loop primers that increase the sensitivity and reduce the reaction time of the assay, the increase in turbidity of positive samples that allows the simple visual assessment of the results, the ability to add fluorescence to augment simple endpoint detection, less sensitivity to inhibitory substances, and the lack of necessity for complicated laboratory equipment or highly skilled staff. The authors then review how LAMP has been applied to the study of infectious diseases and argue that the technique offers a significant opportunity for expanding access to simple, inexpensive, reliable diagnostic tools in socially and economically challenged areas.

Paperna, I. 1984. Reproduction cycle and tolerance to temperature and salinity of *Amyloodinium ocellatum* (Brown, 1931) (Dinoflagellida). Annal. Parasitol. Hum. Comp. 59:7-30.

This paper established the basic morphological and biological characteristics of the parasite and delineated the effects of salinity and temperature on parameters of the life cycle. It was shown that the parasite could exist in a wide range of temperatures and salinities, but that the ideal temperature and salinity 25C and 33 PSU (although salinity tolerance varies with temperature). Deviations from the ideal conditions affected the development and reproductive capacity of the parasite, but the parasite could survive until normal conditions were reestablished and normal reproduction could resume. Thus, the paper demonstrated why *Amyloodinium ocellatum* is should a troublesome pest.

Noga, E. J. 2012. *Amyloodinium ocellatum*. In: Fish Parasites. Pathobiology and Protection. Eds: Woo, P.T.K. & Buchmann, K. CAB International (Cambridge, USA).

This paper gives an overview of the research conducted on *A. ocellatum* focusing on new diagnostic methods developed (specific PCR primers targeting the 3' end of the Large Subunit of the rDNA gene complex), most common internal/external lesions associated with outbreaks (e.g. anorexia, pruritis, gill hyperplasia, etc), protective/control measures, environmental treatments and innate/acquired resistance. This paper concludes that a better understanding of the environmental conditions affecting parasite growth and survival is essential to improve control strategies. Also, it highlights the importance of the development of highly specific and sensitive

tests to detect all the different isolates of the parasites particularly when there is low number of parasites (or latent carriers).

PRE-SPAWNING CAROTENOID FORTIFIED DIETS IMPROVE REPRODUCTIVE TRAITS OF CHANNEL CATFISH, *ICTALURUS PUNCTATUS* AND SUBSEQUENT PROGENY PERFORMANCE

Chatakondi, N

J. Sawanboonchun, W. J. Roy, D. A. Robertson and J. G. Bell. 2008. The impact of dietary supplementation with astaxanthin on egg quality in Atlantic cod broodstock (*Gadus morhua*, L.) Aquaculture 283:97-101

The authors conducted this study to address the sub-optimal levels of carotenoids observed in farmed broodfish and theire effect on their reproductive performance. Hence, this study was conducted to assess the effect of supplementing astaxanthin in broodfish diets to Atlantic cod broodfish for two months prior to spawning. The authors found that supplemental diets increased the concentration of astaxanthin in eggs by 2-3 fold in the farmed brood fish. Higher astaxanthin levels prompted an increase of 20% higher in number of eggs produced and increased the fertilization by 47% compared to the control group. They also postulated that this improvement may be attributed to the efficacy of astaxanthin supplementation in stress reduction to enhance the immune system. The benefits of astaxanthin supplementation to broodstock prompted the authors to suggest that all hatcheries be advised to address the problem of low astaxanthin concentration in the oocytes of pre-spawning broodfish. Implications for nutrient fortification in pre-spawning diets in farmed brood fish be derived from information presented in this paper.

T. Ytrestoyl and B. Bjerkeng, 2007. Dose response in update and deposition of intraperitoneally administered astaxanthin in Atlantic salmon (*Salmo salar* L.) and Atlantic cod (Gadus morhua L.) Aquaculture 263:179-191.

The authors determined the dose-response of astaxanthin in plasma, muscle, liver, kidney and skin after intraperitoneal administration of hgh doses of water dispersible astaxanthin in Atlantic salmon and Atlantic cod. The present study shows that the route of administration has a profound effect on the amount of astaxanthin that is taken up in the blood and deposited in the muscle. The authors suggested that their procedure would increase the concentration of astaxanthin in broodfish by three times compared to the broodfish administered by dietary means. Further, the authors concluded that the limitations observed dietary means of astaxanthin supplementation was not by the limited number of binding sites in the muscle as previously thought rather the limited up-take by the gastrointestinal tract of the fish. The magnitude of the response of Atlantic salmon was higher than that of Atlantic cod. The authors concluded that

high concentration of astaxanthin could be obtained in Atlantic salmon in a short time by intraperitoneal injection compared to supplemental feeding means to have a positive impact on reproductive performance. Implications of carotenoid by intraperitoneal injection in broodfish for rapid incorporation of this nutrient in fish in a short time was presented in this paper.

S. Kim, J. Song, K. Kim and K. Lee. 2012. Effects of dietary astaxanthin on innate immunity and disease resistance against *Edwardsiella tarda* in Olive Flounder *Paralichthys olivaceus.* The Israeli Journal of Aquaculture (Bamidgeh) 64:740-746.

The authors conducted this study with the aim of reducing or preventing the overuse of antibiotics in aquaculture and approached by immunomodulatory effects of nutrients, especially astaxanthin as a dietary supplement. The authors also examined the effect of astaxanthin on sparing vitamin C, non-specific immune response, disease resistance against pathogenic agents and the immunostimulant efficiency in Olive Flounder. The authors conducted a 15-day feeding trial with 0, 1, 2 and 3% Astaxanthin supplemented diet fed in replicated tanks with 30 fish per tank. At the end of the study, the fish from each treatment group were given an intra-peritoneal injection with an *Edwardsiella tarda* suspension and mortalities were recoded daily for 8 days. The authors documented the immune reponse of bacterial disease challenge in the form of non-specific response: Nitro-blue-tetrazolium (NBT) assay, Liver superoxide dismutase (SOD), and vitamin C sparing due to the effect of astaxanthin fortification. The authors concluded that 3% astaxanthin administration enhanced the non-specific immune response and reduced the cumulative mortality to E. *tarda* in Olive Flounder under controlled conditions. Implications for nutrient derived immunity in fish is presented in this paper.

Scanini, V., H. Fernandez-Palacios, L. Robaina, T. Kalinowski and M. S. Izquierdo. 2010. Reproductive performance of gilthead seabream (*Sparus aurata* L) fed two combined levels of cartoenoids from paprika oleoresin and essential fatty acids. Aquaculture Nutrition 17 (3):304-312

The authors fortified broodfish diets with natural sources of carotenoids to assess their effect on reproductive performance of gilthead seabream. Two combined levels of paprika oleoresin at 40 and 60 mg/Kg along with two levels of n-3 HUFA were assessed in triplicate groups of broodfish for 6 weeks. The authors demonstrated that an increase in carotenod content in eggs significantly improved the egg viability, hatching rates and fecundity. The authors concluded that the increased fertility observed in this study was attributed to the antioxidant function of carotenoids, which played a role in protecting sperm cells from lipid peroxidation, enhanced sperm motility and fertility of seabream eggs. Implications for paprika oleoresin supplementation in broodfish diets enhance reproductive performance of seabream.

WITHERING SYNDROME: DISTRIBUTION, IMPACTS, CURRENT DIAGNOSTIC METHODS AND NEW FINDINGS

UJNR Annotated Bibliography

Friedman, C

Research in my laboratory focuses on marine organismal health with a focus on infectious diseases of marine invertebrates and the role of climate drivers (e.g. abalone withering syndrome and oyster herpes virus). In addition to disease, we also evaluate other facilitators of abalone population health and help develop restoration of impacted populations. Below I describe papers that highlight this research with a focus on recent advances.

Withering Syndrome:

Wetchateng,T, Friedman, C.S., , Wight, N.A., Lee, P.-Y., Teng, P.H., Sriurairattana, S., Wongprasert, K., and Withyachumnarnkul, B. 2010. Withering syndrome in abalone *Halioits diversicolor supertexta*. Diseases of Aquatic Organisms 90(1):69-76. (Note that both Wetchateng and Friedman are First authors).

This manuscript documents the first observation of the withering syndrome bacterium in Thailand, Taiwan and the Peoples Republic of China. The paper describes the pathogen and its impact on the host. Importation records combined with samples analyzed suggested that the bacterium was imported into Thailand via live animal transfers from California, USA initially to China and Taiwan. The paper discusses the need for health examinations prior to animal movements and the use of diagnostic methods to reduce the chance of introducing pathogens with animal movements.

Braid, B. A., J. D. Moore, T. T. Robbins, R. P. Hedrick, R. S. Tjeerdema, and C. S. Friedman. 2005. Health and survival of red abalone, *Haliotis rufescens*, under varying temperature, food supply, and exposure to the agent of withering syndrome. Journal of Invertebrate Pathology 89(3):219-231.

Braid et al. (2005) represents the M.S. thesis of Beverly Braid, a graduate student of mine from UC Davis (originally co-advised by Dr. R. Hedrick and me but when I moved to UW, I became a committee member). This paper was one of a two part collaborative project examining withering syndrome between the Tjeerdema, Hedrick and Friedman laboratories:

A key element in understanding the disease physiology involved our discovery that rickettsia-infected abalone require a thermal stress to develop clinical withering syndrome. Although this fact was previously demonstrated, in Moore et al. (2000) we used abalone that had been infected *in situ* and did not examine transmission as well. In Braid et al. (2005), we exposed red abalone to the same does of the withering syndrome rickettsia and examined differential transmission, pathogenesis, and survival at two environmentally relevant temperatures. Only 1.7% of the abalone were infected when held at ambient temperature (12.3^{o}C), while 72-94% of those held at 18.7^{o}C became infected. In addition, we discovered that food availability significantly influenced the proportion of exposed abalone that became infected with the withering syndrome rickettsia; infection intensity was similar between these two treatments. Surprisingly, abalone fed kelp developed more parasite-induced metaplasia than did starved individuals. However, losses were higher in infected, starved abalones than those that were fed. Starved abalone at both

temperatures experienced high mortality than did fed animals and when held at the elevated temperature, the withering syndrome bacterium exacerbated losses induced by starvation and elevated water temperatures (e.g. as during an ENSO event).

This paper also investigated physiological aspects of disease development including mechanisms of cellular change and energy reserves. We determined that depletion of glycogen reserves occurred in the digestive gland and foot muscle prior to pedal catabolism and visible atrophy. In addition, we used two methods to try and identify the mechanism of metaplastic changes observed in the digestive gland and revealed that neither elevated cell proliferation nor apoptosis were involved.

These studies were complemented by a related manuscript using a metabolomic approach to assess biochemical changes in infected and uninfected red abalone (Robenblum et al. (2005). An extension of our investigation into the role of climate change on abalone health is found in:

Vilchis, L.I., Tegner, M.J., Moore, J.D., Friedman, C.S., Riser, K.L., Robbins, T.T., and Dayton, P.K.. 2005. Effects of ocean warming on the growth, reproduction and survivorship of red and green abalones in southern California. Ecological Applications 15(2):469-480.

Red and green abalone were exposed to varying temperature conditions (ambient $\pm$ 3^{o}C) in a withering syndrome endemic zone. When red and green abalone were exposed to thermal stress (ocean warming), red abalone reproduction was compromised green abalone were unaffected. In addition, red abalone are more susceptible to withering syndrome than are green abalone (Vilchis *et al.* 2005). This collaborative research has resulted in a more thorough understanding of the impacts of withering syndrome on abalone and the effects of climatic variation of the potential impact of this disease on abalone populations.

Another significant contribution to our understanding of this disease deals with its control in commercial aquaculture or captive rearing settings through the used of an antibiotic via a series of manuscripts (Friedman *et al.* 2003, 2007, Rosenblum et al. 2005).

Friedman, C.S., Trevelyan, G., Mulder, E.P., and Fields, R. 2003. Development of an oral administration of oxytetracycline to control losses due to withering syndrome in cultured red abalone *Haliotis rufescens.* Aquaculture 224(1-4):1-23.

This research provided data for the compassionate use of the oxytetracycline to clear rickettsial infections in abalone via the US Food and Drug Administration – Center for Veterinary Medicine Program for Investigational New Animal Drugs. We demonstrated that the therapeutant was rapidly depleted from the pedal muscle of medicated abalone and the participating farm was given a 30 day withdrawal period. However, we later investigated differential clearance of oxytetracycline from other tissues and demonstrated that the antibiotic was retained in the digestive gland for prolonged periods (months!) in:

Rosenblum, E.S., Robbins, T.T., Scott, B.B, Nelson, S., Juhasz, C., Craigmill, A., Tjeerdema, R.S, Moore, J.D, and Friedman, C.S. 2008. Efficacy, tissue distribution, and residue depletion of oxytetracycline in WS-RLP infected California red abalone Haliotis rufescens. Aquaculture 277:138-148.

In these experiments, abalone were medicated using 100mg/kg oxytetracycline for varying timer periods to examine not only treatment efficacy and depletion but also protection from re-infection. Levels of oxytetracycline accumulated and retained in abalone digestive gland was far greater than ever reported in the literature in any other animal investigated (marine or terrestrial). Based on this data, the farm mentioned above stopped all treatments. We also examined the level of divalent cations in abalone digestive gland and hypothesized that the observed high metal concentrations may be responsible for the long retention time of this drug in our animals. Using our re-challenge studies we also demonstrated that low levels of oxytetracycline are protective against exposure to the withering syndrome rickettsia, further optimizing the use of this therapeutant in a judicious manner. Although the long depletion time for this drug in abalone is problematic from a commercial culture and environmental stand point, it provides an opportunity for captive rearing of endangered abalone. A single treatment series may protect the animals for months if reared in waters in which the bacterium is present; the withering syndrome rickettsia ranges from Mexico north to San Francisco (Friedman and Finley 2003) and has been found in two locales in northern California where (infected) abalone were out-planted prior to the identification of the causative agent of this disease. In fact, our treatment has been used to protect endangered white abalone broodstock, to reduce losses in captive-bred juvenile white abalones and is being incorporated into the NMFS white abalone management plan as required by the Endangered Species Act (Friedman et al. 2007).

This information not only aids in the control of this disease but has allowed us to better examine the host physiology and pharmacokenetics of this drug in abalone. In fact, abalones appear to have the longest retention of oxytetracycline of any animal examined to date, which is crucial information for the aquaculture industry and US-FDA/CVM, the agency that regulates drug approval in the USA.

Ostreid (Oyster) Herpesvirus

Another important area of research in my laboratory deals with health of the Pacific oyster, an important species within our US west coast aquaculture industry. We have developed a collaborative program with researchers in the USA and France to better investigate causes of oyster mortality with a focus on early life stages and the influence of pathogens and climate on disease expression.

Burge, C.A., Griffin, F.J., and Friedman, C.S. 2006. Summer mortality and herpes virus infections of the Pacific oyster, *Crassostrea gigas*, in Tomales Bay, California. Diseases of Aquatic Organisms 72:31-43.

This collaborative research complements Burge et al. (2007) in which our research team as well as Dr. Renault (IFREMER, France) assessed the influence of site, planting time, oyster line and climatic conditions (temperature and salinity), and phytoplankton assemblages on Pacific oyster survival. The present appear used a combination of field and laboratory studies to evaluate the influence of oyster stock, seed size, site, and oyster herpesvirus (OsHV) presence on survival and growth of Pacific oyster seed. We demonstrated that OsHV was present in moribund oysters and that it's presence predicted seed oyster mortality. In addition, differential losses were observed among oyster stocks planted in the bay. One family produced from wild stock from Washington state, routinely out-performed other commercial stocks or selected family lines employed in this study, thereby providing oyster culturists will important information for sustainability of their industry. Seed size was inversely correlated with survival, while temperature and OsHV presence directly correlated with losses suggesting a synergistic effect of these two stressors on disease expression. We demonstrated that cellular changes suggestive of herpesviruses were rare and suggest that multiple diagnostic methods are needed to examine the influence of OsHV on seed oyster survival.

This paper provided the foundation of our more recent OsHV research designed to better understand the ecology of this disease: we developed a quantitative, real-time PCR assay to detect OsHV in oyster and water samples. Using this assay as well as histological analyses we demonstrated that although viral DNA was detected in several bivalves in Tomales Bay (*C. gigas*, *Ostrea edulis*, *C. virginica*, *C. sikamea*, *Mytilus galloprovincialis*, and *Venerupis phillipinarum*), significantly higher OsHV copy numbers were observed in the Pacific oyster (Burge et al. *in review*). This research is complemented by several other papers by Burge et al. 2007, 2010, 2011.

FEEDING HATCHERY-PRODUCED LARVAE FROM THE GIANT GROUPER *EPINEPHELUS LANCEOLATUS*

García-Ortega, A

Tucker, J.W. and Woodward, P.N.. 1996. Nassau grouper aquaculture. Pp. 363-377. In: F. Arreguin-Sanchez, F., Munro, J.L., Balgos, M.C. and Pauly, D. (eds.) Biology, fisheries and culture of tropical groupers and snappers. ICLARM Conf. Proc. 48. 449 p.

The authors of this paper review multiple spawning, larval rearing and growout trials for the Nassau grouper (*Epinephelus striatus*). They were able to successfully spawn the grouper by multiple methods including combinations of induced (HCG) and natural ovulation along with artificial and natural fertilization. During multiple larval rearing trials using different size tanks, feeding regimes, and other parameters the best survival recorded was 5% from fertilization to 98 dph. During growout grouper reached a mean weight of 1.5 kg at 23 months with a respective

food conversion ratio of 0.9-1.3. At 28 months the mean weight was 2.0 kg with a food conversion ratio of up to 1.8.

Colin, P.L., Koenig, C.C. and Laroche, W.A. 1996. Development from egg to juvenile of the red grouper (*Epinephelus morio*) (Pisces: Serranidae) in the laboratory. Pp. 399-414. In: F. Arreguin-Sanchez, F., Munro, J.L., Balgos, M.C. and Pauly, D. (eds.) Biology, fisheries and culture of tropical groupers and snappers. ICLARM Conf. Proc. 48, 449 p.

This paper represents one of the first successful spawning and larval rearing trials for the red grouper (*Epinephelus morio*). This paper offers an in depth description of larval development of this grouper from egg to juvenile focusing on morphometrics, pigmentation and behavior. Furthermore, this paper describes how salinity and temperature affects growth and development. Eggs averaged 0.95mm diameter and hatched in ~30 hours at 24°C with metamorphosis occurred starting 35 days post hatch at a size of 20 mm standard length.

Kiriyakit, A., Gallardo, W.G. and Bart, A.N. 2011. Successful hybridization of groupers (*Epinephelus coioides* x *Epinephelus lanceolatus*) using cryopreserved sperm. Aquaculture 320:106-112.

In this recent paper the larval rearing of a grouper hybrid from *E. coioides* eggs and *E. lanceolatus* sperm is described. Feeding of hybrid larvae was progressively done using oyster trocophore larvae, rotifers and *Artemia*. This is an important paper because little if any information exists in the larval rearing and development of the giant grouper. In addition, this paper reports rotifers were used without copepods in the successful larval rearing of this hybrid, which was not achieved in our study with *E. lanceolatus*. Additional information is provided on the larval development and metamorphosis of the hybrid fish that in some aspects is similar to the developmental stages we observed during in the larval rearing of *E. lanceolatus*.

STUDY ON HIGH INCIDENCE OF DEATH DUE TO COLLISION OF HATCHERY-REARED PACIFIC BLUEFIN TUNA *THUNNUS ORIENTALIS* JUVENILES IN NET CAGES

Higuchi, K

Miyashita, S., Sawada, Y., Hattori, N., Nakatsukasa, H., Okada, T., Murata, O. and Kumai, H. 2000. Mortality of northern bluefin tuna *Thunnus thynnus* due to trauma caused by collision during growout culture. Journal of the World Aquaculture Society, Vol 31. Pp 632-639.

Collisions with the walls of tanks or nets caused high mortality of northern bluefin tuna during juvenile stage. In this study, juveniles were reared in indoor tanks from 30 to 120 day post-hatching (dph) and in a sea net cage from 42 to 150 dph, and dead fish were examined to detect injury of the bones using x-ray. As a result, more than 98% fish were dead until end of both experiments. The proportion of dead fish with injuries of bone, especially of the vertebral column and the parasphenoid, increased after fish reached 50 mm in BL (35 dph), and exceeded 60% in fish with BL 85 mm (40 dph) or greater in the indoor tanks. Also, the injuries of the vertebral column and the parasphenoid were observed in most of dead fish in the net cage. These results show that the loss of juvenile bluefin tuna was caused by collision with the tank or net wall that fatally damaged the bones of the vertebral column and the parasphenoid.

Masuma, S., Kawamura, G., Tezuka, N., Koiso, M. and Namba, K. 2001. Retinomotor responses of juvenile bluefin tuna *Thunnus thynnus*. Fisheries Science, Vol 67. Pp 228-231.

In bluefin tuna culture, a high mortality of juveniles is caused by collision with the tank and net cage walls at dawn. This collision can possibly be attributed to visually disoriented behavior of the fish. To examine this possibility, the authors carried out retinomotor response experiments with juvenile bluefin tuna and measured ambient light intensity at the culture site at dawn. The light intensity at which the transition from scotopic to photopic vision takes place was 7.52 lx and the time taken by the transition was 15 min. At dawn, the ambient light intensity rapidly increased from scotopic light intensity level and attained photopic light intensity level in 10 min. This incompatibility of the retinal adaptation with the change in the ambient light intensity could cause the visual disorientation of the fish. It is therefore possible that the visually disoriented juveniles cannot control their high power swimming and thus collide with the walls at dawn.

Ishibashi, Y., Honryo, T., Saida, K., Hagiwara, A., Miyashita, S., Sawada, Y., Okada, T. and Kurata, M. 2009. Artificial lighting prevents high night-time mortality of juvenile Pacific bluefin tuna, *Thunnus orientalis*, caused by poor scotopic vision. Aquaculture, Vol 293. Pp 157-163.

In a lab-based study using infrared camera, death of juvenile Pacific bluefin tuna (PBT) was associated with touching and/or colliding with the net wall attached to an indoor tank, then exhibiting abnormal swimming behavior, then becoming moribund during the night and dawn. Also, the scotopic visual threshold of juvenile PBT was at least 40 fold inferior to that of juvenile red sea bream, ocellate puffer, purplish amberjack and grouper. To make up to low scotopic vision sensitivity of juvenile PBT and prevent juvenile PBT from touching and/or colliding with the net wall, the authors illuminated sea net cages by overhead lights providing 200-3000 lx intensity at the water surface. As a result, survival rate of juvenile PBT in the sea net cages significantly improved to 73% up to 23 days post-transfer compared to 12% survival in non-light control net cage.

SUCCESS OF SEED PRODUCTION OF HUMPHEAD WRASSE *CHEILINUS UNDULATUS* WITH IMPROVEMENT OF SPAWNING INDUCTION, FEEDING, AND REARING CONDITION

Hirai, N

Hirai, N., M. Koiso., K. Teruya., M. Kobayashi., T. Takebe., T. Sato., K. Nakamura., T. Goto., and A. Hagiwara. 2012. Rearing conditions for Humphead Wrasse *Cheilinus undulatus* Larvae, and Introduction of the Minute Rotifer *Proales similis* as an Initial Live Food. Journal of Fisheries Technology, Vol 4:2, Pp 57-64. (in Japanese with English abstract)

Fisheries Research Agency and Nagasaki University. 2011. Japan's first production of humphead wrasse fry – World's first reproducible production – (in Japanese), Press Release, URL: http://www.fra.affrc.go.jp/pressrelease/pr23/231129/index.html

Wullur S, Sakakura Y, and Hagiwara A (2009) The minute monogonont rotifer *Proales similis* de Beauchamp: Culture and feeding to small mouth fish larvae. Aquaculture, 293, 62-67.

WITHERING SYNDROME IN ABALONE IN JAPAN

Kiryu, I

Kiryu, I. 2011. Aqua Culture Magazine, Midori Shobo Co. Pp 86.

EFFECTS OF DOCOSAHEXAENOIC ACID AND TAURINE LEVELS IN ROTIFERS ON GROWTH AND SURVIVAL OF LARVAL AMBERJACK *SERIOLA DUMERILI*

Matsunari, H

Yamamoto T, Teruya K, Hara T, Hokazono H, Hashimoto H, Suzuki N, Iwashita Y, Matsunari H, Furuita H, Mushiake K 2008. Nutritional evaluation of live food organisms and commercial dry feeds used for the seed production of amberjack *Seriola dumerili*. Fish Sci 74,1096-1108

To improve the nutritional quality of live foods and dry feeds ordinarily used for the seed production of amberjack *Seriola dumerili*, the nutrient contents of rotifers, *Artemia* nauplii and commercial feeds used in two larval production stations were evaluated. For comparison of the nutrient contents, artificially produced larvae, wild-caught juveniles and wild zooplankton samples were also analyzed. The proportions of 22:6n-3 in the polar lipid of the cultured larvae increased by feeding the dry feeds. The taurine contents of the cultured larvae reflected the

contents of their foods(rotifers < dry feed < *Artemia* nauplii). The taurine content and the proportion of 22:6n-3 in *Acartia* spp. were higher than in foods fed to the larvae. These parameters in the wild juveniles were higher than the cultured ones.

Matsunari H, Hashimoto H, Oda K, Masuda Y, Imaizumi H, Teruya K, Furuita H, Yamamoto T, Hamada K, Mushiake K 2012. Effects of docosahexaenoic acid on growth, survival and swim bladder inflation of larval amberjack *Seriola dumerili*. Aquac Res doi:10.111/j.1365-2109.2012.03174.x

The effect of DHA on the growth performance and survival of larval *Seriola dumerili* during the rotifer feeding period was investigated. Amberjack larvae at 3 day post hatching were fed rotifers enriched with freshwater *Chlorella* (Chlo), a mixture (2:1, v/v) of Chlo and DHA-enriched *Chlorella* (DHA-Chlo), DHA-Chlo, and DHA-Chlo and commercial DHA emulsion, in triplicate for 7 days. The survival rate was improved by the enrichment of rotifers with DHA-Chlo alone, and DHA-Chlo and emulsion. Growth and swim bladder inflation of fish fed rotifers enriched with DHA-Chlo were significantly improved, however, with increased levels of DHA further improvement was not found. The DHA requirement of amberjack larvae is estimated to be 1.5mg/g on a dry matter basis of rotifers.

Matsunari H, Hashimoto H, Oda K, Masuda Y, Imaizumi H, Teruya K, Furuita H, Yamamoto T, Hamada K, Mushiake K 2012. Effect of different algae used for enrichment of rotifers on growth, survival and swim bladder inflation of larval amberjack *Seriola dumerili*. Aquac Int doi:10.1007/s10499-12-9522-8

The effect of algae with different DHA contents used for the enrichment of rotifers on the growth performance, survival, and swim bladder inflation of larval amberjack *Seriola dumerili* was investigated. Rotifers were fed with freshwater *Chlorella vulgaris* containing 3 levels of DHA (rotifer containing DHA 0.04, 0.60, 1.32g $100g^{-1}$) and *Nannochloropsis* (rotifer containing DHA, 0.04g $100g^{-1}$; EPA, 2.54g $100g^{-1}$). The same algae were supplemented to the larval rearing tanks in static condition. The larvae in each triplicate group were fed the enriched rotifers from 3 days post-hatch for 7 days. Growth and survival rate of fish fed the rotifers enriched with *Nannochloropsis* were higher than those of fish fed the rotifers enriched with all three *Chlorella* treatments. Swim bladder inflation was lowest in fish fed the rotifers enriched with *Nannochloropsis*.

Although rotifers enriched with *Nannochloropsis* were effective for the growth and survival, DHA is essential for swim bladder inflation in amberjack larvae.

APPLICATION TO THE GENETIC BREEDING USING GENOMICS INFORMATION IN YELLOWTAIL (*SERIOLA QUINQUERADIATA*)

Ozaki, A

Fuji K, Yoshida K, Hattori K, Ozaki A, Araki K, Okauchi M, Kubota S, Okamoto N, Sakamoto T (2010). Identification of the sex-linked locus in yellowtail, *Seriola quinqueradiata. Aquaculture* 308: 51-55

The sex-determining system of yellowtail (Japanese amberjack), *Seriola quinqueradiata*, is not known. In thisstudy, we identified the sex-linked locus in yellowtail and we characterized the sex-determining system by genetic linkage analysis conducted on 19 female and 19 male progenies from a single family. The associations between phenotypic sex and genotypic data of 71 microsatellite markers selected from yellowtail genetic linkage map were tested. The putative sex-determining locus is located between locus Sequ21 and locus Sequ17 in LG12, and the sex-linked alleles were inherited from the female parent. This result suggests that yellowtail has a ZZ–ZW sex-determining system, and that it would be possible to use these sex-linked markers to discriminate the sexes.

Fuji K, Kai W, Kubota S, Yoshida K, Ozaki A, Aoki J, Kawabata Y, Araki K, Tsuzaki T, Okamoto N, Sakamoto T (2012). A genetic linkage map of yellowtail (*Seriola quinqueradiata*) constructed with microsatellite isolated from BAC-end sequences. *BMC Genomics*: submitting

The Japanese amberjack/yellowtail (*Seriola quinqueradiata)* is a popular marine cultured fish in Japan. Presently, the growing fish pens are stocked with wild-caught juvenile but using cultured brood fish for seed would have less environmental impact. Therefore, it is requested to change the seeds from wild to artificial ones, and allow the selection of commercially important traits. Information on genetic markers and genomic large insert libraries associated with QTL can be used to select individuals carrying desired traits in breeding programs and to identify causative genes. In this study, we prepared a BAC library and present the female and male linkage maps of yellowtail with microsatellite markers obtained from BAC-end sequences (BESs) and a yellowtail genomic library. The BAC library consists of 110,592 clones with an average insert size of 140.7 kb, representing a 16-fold coverage of the genome. From 5,324 BESs, 743 primer pairs were designed to amplify a mapping panel and 373 primer pairs were identified. A total of 464 microsatellite markers derived from a yellowtail genomic library were also mapped. These 837 markers were mapped on female and male maps in 24 linkage groups. The difference of recombination rates between the female and male maps was very small (Female:Male=0.99:1). All linkage groups in the maps indicated that the locations of the sex-specific recombination hot-spots were very different in males and females. Female and male linkage maps of yellowtail were constructed using BAC-clones information. After a genomic region has been shown to be linked to trait of economic importance, this high quality BAC library resource and mapped BAC clones information are crucial in the identification and functional characterization of the genetic variation.

Ozaki A, Yoshida K, Fuji K, Kubota S, Kai W, Suzuki J, Akita K, Aoki J, Kawabata Y, Nakagawa M, Hotta T, Tsuzaki T, Okamoto N, Araki K, Sakamoto T (2012). Quantitative trait loci (QTL) associated with resistance to a parasitic disease (Benedenia seriolae) in yellowtail (*Seriola quinqueradiata*) through genome wide analysis. *Heredity*: submitting

Benedenia disease caused by the ectoparasite *Benedenia seriolae* is a serious parasitic disease in marine aquaculture finfish, leading to secondary viral or bacterial infections. Because fish rub their bodies against the fish cage to remove the parasite, the mortality is quite high especially in juveniles. Also the method to remove requires a lot of time, cost and effort. Benedenia disease is difficult to prevent in marine aquaculture systems. Genetic variation has been indicated to play a significant role to determining the susceptibility to this parasitic disease, however, the mechanisms involved in the differential response to infection remain poorly understood. To evaluate the genetic basis of Benedenia disease resistance in yellowtail (*Seriola quinqueradiata*), a genome-wide and chromosome-wide linkage analysis was initiated using F1 yellowtail families. Two major quantitative trait loci (QTL) regions on linkage groups Squ2 and Squ20 were identified and then confirmed in F1 families. These QTL regions explained 32.9–35.5% of the phenotypic variance. On the other hand, the QTL related to growth was found in other linkage groups (Squ7). Therefore, Benedenia disease resistance QTL was not correlated with fish size. At the results, we have discovered the first genetic evidence that contributes to detailing the phenotypic resistance to Benedenia disease, and the results will help resolve the mechanism of resistance to this important disease of yellowtail.

THE COOPERATIVE CULTURE OF SEAWEED IN NEW ENGLAND—HOW RESEARCH, INDUSTRY, AND EXTENSION ARE CULTIVATING A NEW FIELD IN AQUACULTURE

Redmond, S

Benson, Judy. 2012, March 11. Seaweed farming in the Sound: The beginning of something big? The Day. Retrieved from http://www.theday.com/article/20120311/NWS01/303119895/1018

This Connecticut newspaper article summarizes the research work on native New England seaweed species for farms in Maine and Long Island Sound. A collaborative research project involving the University of Connecticut Marine Biotechnology Research Laboratory and commercial partners Ocean Approved, Llc in Maine resulted in the first successful kelp culture crops being cultivated both in Maine and Long Island Sound in the winter of 2011, with plans to further develop farming operations in the future.

Maine Sea Grant. 2012. Seaweed Production on Mussel Farms in Maine:

UJNR Annotated Bibliography

A Pilot Project to Stimulate Seaweed Production on Mussel Farms in Maine [Press Release] Retrieved from http://www.seagrant.umaine.edu/extension/kelp-mussels

This online press release from Maine Sea Grant summarizes the collaborative research work conducted by shellfish growers, seaweed farmers, and marine extension agents in Maine. The project was the first of its kind in Maine, integrating Saccharina latissima on six different shellfish farms (mussel and oyster) in Maine.

Morrissey, MT & S Almonacid. 2005. Rethinking technology transfer. *Journal of Food Engineering*, Volume 67, Issues 1–2, pp. 135-145. (http://www.sciencedirect.com/science/article/pii/S0260877404003346)

This article uses several new seafood processing innovation examples to highlight the importance of a new approach to technology transfer. In order to improve and compete in a global marketplace, businesses need more real-time, adaptable approaches to technology and research being developed in labs and Universities. In order to keep the speed of transfer up with the speed of new technology development, the authors suggest a more multi-disciplinary and dynamic approach.

Warner, KD. 2008. Agroecology as Participatory Science: Emerging Alternatives to Technology Transfer Extension Practice. *Science, Technology, & Human Values* , Vol. 33, No. 6, pp. 754-777.

Agricultural extension plays an important role of linking the research community with the business community, transferring new technologies from scientists to farmers, and problems of farmers as research questions back to scientists. Besides just technology transfer, extension plays a wider role as educators of the general public, integrating new technologies, producers, consumers, and issues arising from these. This article reviews these interactions by looking at environmental pollution issues and agroecological partnerships between growers, extension, and scientists. This type of participatory science uses collaboration, multidisciplinary approaches, and ecological approaches to improve the direction of agricultural developments.

Parkhurst, Emily. 2011, September 26. Kelp wanted: Seaweed farms expand in Maine's Casco Bay. The Forecaster. Retrieved from http://www.theforecaster.net/content/pnm-kelp-farm-near-chebeague-092811

This newspaper article reports on the expansion of the first commercial kelp farm in the United States, Ocean Approved, Llc, in Maine. They are a new company with two years of cultivation experience, with plans to expand their product lines and further develop seaweed aquaculture in Maine. Part of their plan involves sale of their native "seed" plants to other growers in Maine.

INTENSIVE JUVENILE PRODUCTION OF YELLOWTAIL AMBERJACK (*Seriola lalandi*) IN SOUTHERN CALIFORNIA

Rotman, F

Attramadal, K.J.K., T. Bjørnar, I. Salvesen, G. Øie, O. Vadstein and Y. Olsen. 2012. Ceramic clay reduces the load of organic matter and bacteria in marine fish larval culture tanks. Aquacultural Engineering Volume 49 Pp 23-34.

The authors of this paper describe how the use of ceramic clay as a replacement for live microalgae or algae pastes can increase turbidity without contributing to the organic matter load in larval marine fish rearing systems. A series of experiments is described that demonstrate encouraging results of the larval performance of Atlantic cod (*Gadus morhua*) reared with clay as a turbidity source compared to those reared using standard live microalgae and paste protocols.

Hernández-López, J., M.A. Guzmán-Murillo and F. Vargas-Albores. 1995. Quantification of pathogenic marine *Vibrio* using membrane filter technique. J. of Microbiological Methods Volume 21:2 Pp 143-149.

These authors describe a rapid and simple methodology based on a membrane filter technique designed to detect and quantify marine *Vibrio* spp involved in fish and shellfish diseases as well as human gastrointestinal disorders. This technique can be instrumental in the management of bacterial communities in marine larval fish culture systems.

Olafsen, J.A. 2001. Interactions between fish larvae and bacteria in marine aquaculture. Aquaculture, Volume 200 Pp 223-247.

This publication is an excellent review of the influence bacterial ecology can have on the culture of marine organisms. Specifically, the author reviews the interaction between various bacterial species and larval marine fish. Understanding concepts discussed in this publication are instrumental to the successful larval rearing of various marine organisms.

Yamamoto, T., K. Teruya, T. Hara, H. Hokazono, H. Hashimoto, N. Suzuki, Y. Iwashita, H. Matsunari, H. Furuita and K Mushiake. 2008. Nutritional evaluation of live food organisms and commercial dry feeds used for seed production of amberjack *Seriola dumerili*. Fisheries Science, Volume 74 Pp 1096-1108.

The authors of this influential paper compare the nutritional profiles of both wild plankton and wild *Seriola dumerili* juveniles to commercially produced dry diets, cultured live feeds and cultured juvenile fish. Differences in resulting nutrient levels help to identify potential deficiencies in cultured *S. dumerili*. More specifically, it was found that 22-6n-3 and taurine are much higher in wild copepods (*Acartia* spp.) and *S. dumerili* juveniles than the cultured counterparts of both.

IN SITU SWIMMING AND SETTLEMENT BEHAVIOR OF CULTURED SERRANID LARVAE, *PLECTROPOMUS LEOPARDUS* AND *EPINEPHELUS MALABARICUS*

Shibuno, T

Masuma, S., N. Tezuka and K. Teruya. 1993. Embryonic and morphological development of larval and juvenile coral trout, *Plectropomus leopardus*. Japanese Journal of Ichthyology, 40(3): 333-342.

At the Japan Sea Farming Association, Yaeyama Station, coral trout broodstock have provided fertilized eggs by spontaneous spawning since May 1988. They described the embryonic and morphologica1 development, behavior, and growth of the coral trout from the egg to the juvenile stages.

Leis, J. M. and B. M. Carson-Ewart. 1999. In situ swimming and settlement behavior of larvae of an Indo-Pacific coral-reef fish, the coral trout *Plectropomus leopardus* (Pisces: Serranidae). Marine Bio1ogy134: 51-64.

Late larvae of the serranid coral trout *Plectropomus leopardus* (Lacepède), captured in light traps, were released during the day both in open water and adjacent to two reefs, and their behaviour was observed by divers at Lizard 1sland, northern Great Barrier Reef.

Nakai, T. 2002. Management of fishery resources for groupers (Serranidae) in Okinawa, southern Japan. Fisheries Science, 68(Supplement): 431-432.

They show data on the distribution of groupers across the coral reef area at Iriomote Island, and a summary on the seed production of several species of grouper in the Ryukyu Island, Okinawa. Their data on the distribution of groupers provide additional information on the management strategy for groupers.

Shibuno, T., Y. Nakamura, M. Horinouchi and M.Sano. 2008. Comparison of reef fish community structures from mangrove estuary to coral reef slope, at Ishigaki Island, southern Japan. Ichthyological Research. 55(3):218-237.

To clarify seascape-scale habitat use patterns of fishes in the Ryukyu Islauds (southern Japan), visual censuses were conducted in the mangrove estuary, sand area, seagrass bed, coral rubble area, branching coral area on the reef flat, and tabular coral area on the outer reef slope.

EFFECTS ON GROWTH OF FLATFISH JUVENILE BY ARTIFICIAL LIGHTENING CONDITIONS: INTENSITY, PHOTOPERIOD AND WAVELENGTH

Shimizu, D

Itoh, K., Y. Washio, Y. Fujinami, D. Shimizu, S. Uji, H. Yokoi, T. Suzuki. 2012. Continuous illumination through larval development suppresses dopamine synthesis in the suprachiasmatic nucleus, causing activation of α-MSH synthesis in the pituitary and abnormal metamorphic skin pigmentation in flounder. General and Comparative Endocrinology 176: 215–221.

In order to better understand the endocrine aberrations related to abnormal metamorphic pigmentation that appear in flounder larvae reared in tanks, this study examined the effects of continuous 24-h illumination (LL) through larval development on the expression of *tyrosine hydroxylase-1* (*th1*), proopiomelanocortin (pomc), α-melanophore-stimulating hormone (α-MSH) and melanin concentrating hormone (MCH), which are known to participate in the control of background adaptation of body color. We observed two conspicuous deviations in the endocrine system under LL when compared with natural light conditions (LD). First, LL severely suppressed *th1* expression in the dopaminergic neurons in the anterior diencephalon, including the suprachiasmatic nucleus (SCN). Second, pomc and α-MSH expression in the pars intermedia melanotrophs was enhanced by LL. Skin color was paler under LL than LD before metamorphic pigmentation, and abnormal metamorphic pigmentation occurred at a higher ratio in LL. We therefore hypothesize that continuous LL inhibited dopamine synthesis in the SCN, which resulted in up-regulation of pomc mRNA expression in the melanotrophs. In spite of the up-regulation of pomc in the melanotrophs, larvalskin was adjusted to be pale by MCH which was not affected by LL. Accumulation of α-MSH in the melanotrophs is caused by uncoupling of α-MSH synthesis and secretion due to inhibitory role of MCH on α-MSH secretion, which results in abnormal metamorphic pigmentation by affecting differentiation of adult-type melanophores. Our data demonstrate that continuous illumination at the post-embryonic stage has negative effects on the neuroendocrine system and pituitary in flounder.

LARVAL REARING ADVANCEMENTS FOR YELLOWTAIL AMBERJACK (*Seriola lalandi*) IN SOUTHERN CALIFORNIA

Stuart, K

Attramadal K.J.K., B. Tondel, I. Salvensen, G. Oie, O. Vadstein, Y. Olsen. 2012. Ceramic clay reduces the load of organic matter and bacteria in marine fish larval culture tanks. Aquaculture Engineering 49: 23-34.

The authors present information on the use of ceramic clay as a turbidity source for marine larvae. In contrast to live microalgae or algae pastes, clay increases turbidity without contributing to the organic matter load. The larvae, algae, and live feed increase the microbial carrying capacity of the rearing water which allow exponential growth of bacteria and favor fast-

growing opportunists. Reducing substrate levels by replacing microalgae with clay may reduce bacteria proliferation and benefit larvae. The authors compared the effects of three rearing regimes including live *Isochrysis galbana*, *Nannochloropsis oculata* paste, and ceramic clay on the bacterial community, concentration of organic matter, and growth and survival of Atlantic cod larvae (*Gadus morhua* L.). The application of clay resulted in reduced substrate levels for bacteria in the rearing water compared to the addition of live algae or algae paste. The tanks receiving clay showed a lower bacterial level in the water than tanks that added algae paste or live algae. The algae paste treatments showed a higher abundance of bacteria and a higher share of cultivable bacteria and TCBS counts than the other two treatments. Tanks with live algae showed low relative abundances of opportunistic bacteria and TCBS counts in both water and rotifers. Larval growth was significantly higher in the clay and live algae treatments than the algae paste treatment. The survival of larvae in the tanks added clay was variable. Two of the three tanks with clay had significantly higher larval survival than the tanks with live algae or algae paste. However, one tank with clay underwent 100% mortality. The authors could not explain this variation but mention that the effects of clay addition on larval performance should be studied further. Clay addition appears to be an easy way to reduce bacterial load during early first feeding of marine larvae without compromising the beneficial effects of turbidity.

Cook, M.A., R.B. Johnson, P. Nicklason, H. Barnett, M.B. Rust. 2008. Marking live feeds with inert metal oxides for fish larvae feeding and nutrition studies. Aquaculture Research 39: 347-353.

The authors used yttrium oxide (Y_2O_3), ytterbium oxide (Yb_2O_3), lanthanum oxide (La_2O_3) and dysprosium oxide (By_2O_3) as potential live feed markers for feeding and nutrition trials with fish larvae. These markers were evaluated by determining the uptake and depletion of markers over time and quantifying ingestion of yttrium oxide marked rotifers by Atlantic cod (*Gadus morhua*). The authors found that *Artemia* nauplii and rotifers quickly took up markers within 10 minutes and that there was no significant difference among temperatures in depletion of markers (10, 15, and 20 C) with *Artemia* or rotifers. Depletion from rotifers was not significantly different between 5 and 20 minutes or between 5 and 30 minutes for *Artemia*. The authors were able to show that visual *Artemia* estimates consumed by the larvae were similar to consumption estimates determined by analysis of yttrium oxide marked *Artemia* using inductively coupled plasma optical emission spectroscopy.

Stuart, K.R. and M.A. Drawbridge. 2012. Captive spawning and larval rearing of California yellowtail (*Seriola lalandi*). Aquaculture Research doi:10.1111/j.1365-2109.2011.03077.

The authors document spawning patterns, measures of egg production, population fecundity, and egg and larval quality over a four year period for a captive population of *Seriola lalandi*. Spawned eggs were also used to document larval development and to develop rearing

techniques for aquaculture in the region. Authors showed that spawning occurred naturally in the 140 m^3 tank when the ambient water temperature reached 16°C and ended when the temperature exceeded 22°C and egg production reached a maximum when 43 spawn events were recorded from a pool of nine females yielding 36.8 million eggs total. Larval rearing trials yielded survival rates as high as 5.8% from egg to 50 dph. Successful larval culture methods included the addition of algae paste for green water culture, rotifers (20 rotifers ml^{-1}) at 2 dph and *Artemia* (5 *Artemia* ml^{-1}) at 6 dph. Larvae were transferred from the incubation tank at 10 dph to a shallower tank with 33% greater surface area in order to accommodate the larvae's strong orientation to surface waters.

Benetti, D. 1997. Spawning and larval husbandry of flounder (*Paralichthys woolmani*) and Pacific yellowtail (*Seriola mazatlana*), new candidate species for aquaculture. Aquaculture 155, 307-318.

This paper describes the spawning and larval rearing of two new species for aquaculture in Ecuador. Experimental production of these species has been conducted for the first time from eggs in captivity. Survival of *Seriola* throughout larval rearing ranged from 0 – 70%, however high mortalities caused by diseases, cannibalism, and weaning onto artificial diets during and after metamorphosis reduced average survival rates through the juvenile stage to less than 1%. The basic broodstock and larval rearing information provided by the author is a good starting point for the culture of either *Paralichthys* or *Seriola* culture.

Moran, D., C.K. Smith, B. Gara, C.W. Poortenaar. 2007. Reproductive behaviour and early development in yellowtail kingfish (*Seriola lalandi* Valenciennes 1833). Aquaculture 262, 95-104.

The authors describe the spawning behaviour of wild caught brood stock as well as early egg and larval development of yellowtail kingfish (*Seriola lalandi*). Spawning occurred naturally when water temperature was above 17 C. Courtship behaviour involved one male and female, and consisted of a high-speed pursuit punctuated by stalling, nipping and touching. This lasted for approximately 0.5 – 1.5 h until, immediately prior to spawning, the male would nip at the female gonoduct, presumably to induce spawning. Spawning occurred in the early daylight hours at the start of the spawning season, but shifted to around dusck in the latter part. Spawned eggs were positively buoyant, had a high fertilization rate (>99%), ranged 1.33 – 1.50 mm in diameter with a single oil droplet 0.30 – 0.33 mm diameter. Information given in this paper is indicative of a healthy spawning population of *S. lalandi* and can be used as a reference or baseline for culture of this species.

Papandroulakis, N., C.C. Mylona, E. Maingot, P. Divanach. 2005. First results of greater amberjack (*Seriola dumerili*) larval rearing in mesocosm. Aquaculture 250, 155-161.

The authors apply the mesocosm method for larval rearing to the greater amberjack (*Seriola dumerili*). This paper gives a good description of larval rearing for *Seriola* in terms of

weaning schedule, turbidity levels, and live feed levels. Along with the description of the rearing protocols, growth information is provided which is a good template for most *Seriola* species.

Sakakura, Y. and K. Tsukamoto. 1999. Ontogeny of aggressive behaviour in schools of yellowtail, *Seriola quinqueradiata*. Environmental Biology of Fishes 56, 231-242

The authors studies ontogenetic changes in social interactions, especially in aggressive behaviour of the yellowtail, *Seriola quinqueradiata*, and compared these to morphological and physiological changes. No agonistic interactions were observed during the larva period until 10 mm in total length (TL) at approximately 20 days after hatching. Typical shivering behaviour with 'J-posture' was observed during metamorphosis, when fin rays and calcification of vertebra were completed and there was an increase of tissue thyroid hormone. The onset of aggressive behaviour was just after metamorphosis to the juvenile period, and coincided with a significant increase in tissue cortisol levels. The onset of schooling behaviour was at 12 mm TL, slightly after the onset of aggressive behaviour. The authors provided a good basis for determining aggressive behaviour in *Seriola quinqueradiata* which can be used as a model for other *Seriola* species. This paper also presents possible solutions to aggressive interactions found in the culture environment.

Yoseda K., K. Yamamoto, K. Asami, M. Chimuea, K. Hashimoto, S. Kosaka. 2008. Influence of light intensity on feeding, growth, and early survival of leopard coral grouper (*Plectropomus leopardus*) larvae under mass-scale rearing conditions. Aquaculture 279, 55-62.

This study investigated the effect of different light intensities on feeding, growth, and survival of early stage leopard coral grouper *Plectropomus leopardus* larvae. Four different light intensities (0, 500, 1000, and 3000 lx) were used and larvae were kept under constant light conditions from 0 day after hatching (DAH) to 5 DAH. The results indicate that coral grouper larvae are visual feeders and their food intake increases with increasing light intensity and that light intensity is the factor affecting larval feeding, growth, and survival. The authors provide a sound experimental design and interesting results which can translate to any species whose larvae are visual predators. The comprehension of optimization of environmental rearing conditions such as light intensity as it relates to first feeding larvae is necessary in establishing stable mass-scale rearing technology for any species.

EXPERIMENTAL STUDY ON BROODSTOCK MANAGEMENT OF BARFIN FLOUNDER UNDER THE CONCEPT OF MINIMUM KINSHIP SELECTION

Suzuki, S

Nobuhiko Taniguchi. 2004. Broodstock management for stock enhancement programs of marine fish with assistance of DNA markers (a review). Stock Enhancement and Sea Ranching. developments, pitfalls and opportunities (2nd Ed.). Pp 329-338.

The microsatellite DNA markers have been used, and the availability of the markers was recognized in some examples of studies on the genetic divergence and broodstock management of red sea bream for enhancement programs in Japan. Based on these microsatellite DNA data, genetic variability, effective population size and inbreeding coefficients could be estimated for broodstocks in hatchery. A procedure for broodstock management is proposed to maintain the genetic variability of the target fish species. If the genetic divergence is decreased in the brood stock, the minimal-kinship criterion on relatedness estimator may be applied to recover the genetic diversity of the founder population by selective breeding based on this criterion. This procedure might be useful not only in the case when low numbers of parental fish are available, but also in the case of conservation of genetic variability in threatened species.

IMPROVING THE HATCHERY OUTPUT OF THE HAWAIIAN PINK SNAPPER, *PRISTIPOMOIDES FILAMENTOSUS*

Tamaru, C

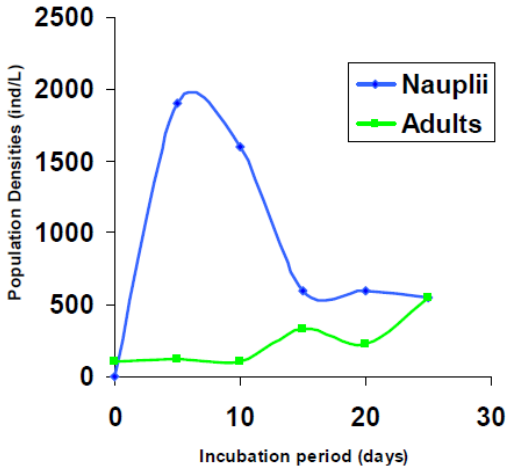
Morote1, E., M.P. Olivar, P.M. Pankhurst, F. Villate, and I. Uriarte. 2008. Trophic ecology of bullet tuna *Auxis rochei* larvae and ontogeny of feeding-related organs. Mar. Ecol. Prog. Ser. 353: 243–254, doi: 10.3354/meps07206

This is a comprehensive study on the diet of early fish larvae of a tuna species collected from the Mediterranean. Assessment of diet was done through gut content analysis in larvae ranging from approximately 2 mm to > 5 mm. In addition, the authors analyzed the development of the digestive system and the eye. Both digestive capacity and visual acuity are changing rapidly in these early larvae, and thus impact what they are capable of seeing, catching and digesting. In terms of diet, the two important conclusions of the authors are: 1) even the smallest larvae ingest a large variety of prey items; 2) size of prey increases with size; and 3) feeding is selective and changes with fish size. These types of ecological studies provide a basis for trying different food types in captivity. However, there are limitations to gut content analysis, and these need to be considered. In particular, small and soft bodied prey may be underestimated in guts due to their rapid digestion.

Herbing, I.H. von, S.M. Gallager, W. Halteman. 2001. Metabolic costs of pursuit and attack in early larval Atlantic cod. Mar. Ecol. Prog. Ser. 216: 201-212

This laboratory-based study provides a cost-benefit analysis for feeding in early larvae of the Atlantic cod. Two prey items are contrasted: copepod nauplii (*Pseudodiaptomus* sp.) and

protozoans (*Balanion* sp.) in 3 sized classes of larvae (5-6, 6-7 and 7-8 mm). The behavioral analysis in this study underscores the differences in the capture of an evasive vs. a non-evasive prey using both respiration rates and swimming speed analyses. Smallest fish larvae were not able to capture copepod nauplii, but fed readily on protozoans. The main conclusion of this study is that a smaller prey is less costly to catch, but also provide much lower benefit. The larger prey (copepod nauplii) required 2-3x more energy to capture, but provided two orders of magnitude greater benefit it terms of calories.



VanderLugt, K. ,M. J. Cooney, A. Lechner, and P. H. Lenz.. 2009. Cultivation of the Paracalanid Copepod, *Bestiolina similis* (Calanoida: Crustacea). World Aquaculture Society, Volume: 40(5):616-628.

VanderLugt, K., B. Clauberg, P. Lenz. 2005. Intensive Cultivation of the Calanoid Copepod *Bestiolina similis.* Volume 12, Issue 4 on 01 April 2005. http://www.jyi.org/research/re.php?id=147

The two references above summarizes the work done as part of a Master's Dissertation and documented the temporal changes in production in batch cultures of the calanoid copepod, *Bestiolina similis*. The focus of the research was to develop management strategies for the use of copepod nauplii as a live food. Population abundances and female egg production rates were measured, and recruitment and mortality rates were calculated. The copepod, *B. similis* fed with three phytoplankton strains: *Chaetoceros neogracille, Isochrysis galbana* Tahitian strain, and *Rhodomonas sp.* at various concentrations resulted in highest survival rates with *I. galbana* at a concentration of 5.0 x 10^4 cells ml^{-1}. However, when copepods were isolated from each culture every 12 hours and egg production monitored, it was also found that copepods fed with *I. galbana* had the highest egg production. The highest average egg production rate was 23 eggs $female^{-1}$ day^{-1} with the maximum measured at 30 eggs $female^{-1}$ day^{-1}. The most intriguing observation from a culture standpoint was that the production cycle was highly predictable (Figure 1) and provided an opportunity to coordinate nauplius production rates with first feed needs of fish larvae. These observations would be replicated in 3,000-L larval rearing tank that would result in high survival during the first feeding stage of the pink snapper.

Figure 1. Temporal changes in nauplii and adult copepod density in response to an *I. galbana* diet.

VanderLugt, K. and P.etra Lenz. 2009. Handbook for the Cultivation of two Hawaiian

Paracalanid Copepods. Technical Report: Pacific Biosciences Research Center. University of Hawaii at Mano. 19 pp.

This technical handout, that is available on the internet, is an excellent compilation of the biology, morphology, identification and step by step description of the techniques of the culture process for the calanoid copepod, *Bestiolina similis*. The descriptions include collection of copepods from the wild and how they are sorted and processed for culture. Information also covers the production of phytoplankton cultures needed to feed the copepods. The guides are useful for small scale culture processes.

UNDERSTANDING *ARTEMIA* BIOGEOGRAPHY RELATED TO HATCHERY PRODUCTION AND JUVENILE QUALITY

Torrentera, L

Seenivasan C., C., P. Saravana Bhavan, S. Radhakrishnan, R. Shanthi. 2012. Enrichment of *Artemia* nauplii with *Lactobacillus sporogenes* for Enhancing the Survival, Growth and Levels of Biochemical Constituents in the Post Larvae of the Freshwater Prawn *Macrobrachium rosenbergii*. *Turkish Journal of Fisheries and Aquatic Sciences. 12: 23-3*

The authors in this study designed and experiment to prove that *Artemia* could be used as vector to deliver probiotics to improve larva digestion and growing. They used *Artemia franciscana* naupli enriched with bacterium, *Lactobacillus sporogenes* and fed the freshwater prawn, *Macrobrachium rosenbergii* post larvae (PL). *Artemia* was enriched with different concentrations of *L. sporogenes*. Enriched *Artemia* produced significantly ($P<0.05$) higher survival and growth when compared with the control fed with un-enriched *Artemia*. *M. rosenbergii* (PL) biomass increase, total weight gain, specific growth rate increase, condition factor and mean conversion ratio were found to be higher in PL fed with *L. sporogenes* enriched *Artemia* when compared with control. The enhanced growth performance of PL fed with *L. sporogenes* was further confirmed by the lower food conversion ratio recorded. The levels of biochemical constituents, such as total protein, amino acids, carbohydrate and lipid contents were found significantly higher ($P<0.05$) in *M. rosenbergii* PL fed on *L. sporogenes* enriched *Artemia* particularly when compared to the un-richer control. The authors concluded that cells *of L. sporogenes* using *Artemia* as a vector can be considered as suitable probiotic for attaining good survival and growth *of M. rosenbergeii* post Larvae.

Soltanian Siyavash, Jean Dhont, Patrick Sorgeloos, Peter Bossier. 2007. Influence of different yeast cell-wall mutants on performance and protection against pathogenic bacteria (*Vibrio campbellii*) in gnotobiotically-grown *Artemia*. *Fish & Shellfish Immunology* 23: 141-153

The present study determined the selection of isogenic yeast strains (with deletion for genes involved in cell-wall synthesis) to evaluate their nutritional and immune-stimulatory characteristics for gnotobiotically-grown *Artemia*. A set of experiments showed the nutritional value of isogenic yeast strains (effected in mannoproteins, glucan, chitin and cell-wall bound protein synthesis) for gnotobiotically-grown *Artemia*. Yeast cell-wall mutants were always better feed for *Artemia* than the isogenic wild type (WT) mainly because they supported a higher survival. The difference in *Artemia* performance between WT and mutants feeding was reduced when stationary-phase grown cells were used. These results suggest that any mutation affecting the yeast cell wall make-up is sufficient to improve the digestibility in *Artemia*. The second set of experiments, investigates the use of a small amount of yeast cells in gnotobiotic *Artemia* to overcome pathogenicity of *Vibrio campbellii* (VC). Among all yeast cell strains used in this study, only mnn9 mutant yeast (less cell-wall bound mannoproteins and more glucan and chitin) seems to completely protect *Artemia* against V C. The result with these mutants is of particular interest, as its nutritional value for *Artemia* as comparable to the wild type. The results of this study suggest non-interference of *Artemia* general nutritional effects.

Shadrin Nickolai, Elena Anufriieva, Ekaterina Galagovets. 2012. Distribution and historical biogeography of *Artemia* leach, 1819 (Crustacea: Anostraca) in Ukraine. *International Journal of Artemia Biology.* Vol. 2, No 2: 30-42

In this study the authors described the recent information about the biogeography of *Artemia* in Ukraine (Ukraine separated from the Soviet Union after the cold war). The authors identified the following areas with potential for aquaculture: The Crimean peninsula; the coastal zone of the NW Black Sea; the north coast of the Sea of Azov; Donetsk Oblast; Zakarpattia Oblast. These authors recognized that at least there are two bisexual species (*A. salina* and *A. urmiana*) and the parthenogenetic complex populations of *Artemia* dwell in the Ukraine. In some lakes (Koyashskoye, Terecly-Konradskoye) they found *A. urmiana* and parthenogens both, but separated in time. In the waters of the Crimea and NW part of the Black Sea brine shrimp are found under salinities from 10-20 ppt (if no other animals present) to 350 - 370 ppt. Larvae and adults of some Coleoptera, Hemiptera, ostracods *Eucypris inflata* (Sars) were found, and probably other invertebrates may eat away *Artemia* completely in the Crimean lakes whose salinity is below 60-110 ppt. Exploitation of salt can create new habitat for brine shrimp. Ukraine hypersaline ecosystems are very promising opportunities for industrial harvesting of *Artemia* cysts.

Cohen Rosa Graciela. 2012. Review of the biogeography of *Artemia* Leach, 1819 (Crustacea: Anostraca) in Argentina. *International Journal of Artemia Biology* Vol. 2, No 1: 9-23.

In this study the author make a review of the biogeography of *Artemia* in Argentina and other countries in South America. She describes two species of *Artemia* in these countries: *A. franciscana* widely distributed throughout the Americas and the Caribbean, and *A. persimilis* with a more restricted distribution in southern South America (Argentina, Chile, Peru, and Bolivia). In Argentina *A. franciscana* is located in the Buenos Aires Province. *A. persimilis*, from: La Pampa and Santa Cruz Provinces. The southernmost record of the species corresponds to Laguna de los Cisnes in the Chilean Tierra del Fuego. Temperature is considered as a main factor in the determination of the present geographic distribution of both species in these countries. *A. franciscana* and *A. persimilis* are considered as endemic species to the Americas. However, in recent decades, *A. franciscana* was intentionally introduced in several countries of the New and the Old World, mainly associated with aquaculture. Two African populations recently were assigned as *A. franciscana* suggesting other natural events raising the hypothesis that the original geographic distribution of this species was not only restricted to the Americas. On the other hand, *A. persimilis* was described for the first time in Salinas Grandes de Hidalgo, Argentina and in Europe from the Italian population of Saline di San Bartolomeo, Sardinia. *A. persimilis* (now extinct) co-occurred with *A. salina* in the later location. The presence of several *Artemia persimilis* populations at high-altitude (above 4,500 m) in the South America Altiplano could lead to an hypothesis that suggest that in the geological past, *A. persimilis* would have retreated to cooler and higher altitudes and latitudes, leaving isolated populations in Andean enclaves.

Torrentera Laura and Stanley I. Dodson. 2004 Ecology of the brine shrimp *Artemia* in the Yucatan, Mexico, Salterns. *Journal of Plankton Research*. Vol 26:(5) 1-8.

In this article the authors described the biogeography and ecology of the brine shrimp *Artemia* across the Yucatan peninsula. They recorded monthly field data during one year at the North Coast hypersaline ecosystems: temporary pond, pools, salterns and saltmarshes. The Yucatan Peninsula is a great limestone platform projecting from Mexico and Central America northward into the Gulf of Mexico and the Caribbean Sea. The most important ports sampled included: Celestun, at the extreme north west of the peninsula and Chuburna and, Xtampu, at the intermediate area and, Las Coloradas, at the extreme northeastern part of the peninsula. The salinity ranges from that of seawater (35 g/L) to about 150 g/L or more. The locations revealed high extreme alkalinity, salinity and temperature conditions, and hypoxia and in some instances, total desiccation. The four locations were similar in water temperature, nitrites, and phosphates, but differed in salinity, oxygen content, water depth, nitrates, ammonia, silicates, carbonates, sulfates, and pH. The dominant multicellular organism in these habitats is *Artemia*, the brine shrimp. *Artemia* abundance and population dynamics were significantly correlated with specific environmental conditions. The annual natural production mean value of Biomass calculated in the sampled area was 450 D. W. (g/square meter). The cyst production mean D. W. value was 733 (g/square meters). The field study provides ecological basis for the exploitation of *Artemia* as a live food that possess good nutritional value for larvae culture of finfish and crustaceans.

These hypersaline ecosystems should be protected because they are nesting and feeding areas for the Caribbean flamingo and other marine birds.

CONDITIONING TECHNOLOGIES FOR FLATFISH STOCK ENHANCEMENT: GLOBAL PROGRESS AND PITFALLS

Walsh, M

Walsh, M. L., E. A. Fairchild, N. Rennels, S. C. Farina, W. H. Howell, R. Mercaldo-Allen, and C. Kuropat. 2009. Rearing Diets for Winter Flounder Optimize Weaning Success in Hatchery, Wild. Global Aquaculture Advocate. May/June: 48-50.

The objectives of this work were to quantify feeding-related performance of reared-then-released winter flounder juveniles that were reared on different feeds (both live and formulated) in the hatchery. The authors evaluated how feeding history translated to wild feeding performance once individuals were released into nature (caged in-situ) by examining survival, growth, feeding onset and incidence, stomach fullness, diet composition, and nucleic acid-based condition. Fish raised on live diets exhibited higher survival and growth than those reared on formulated, pellet feeds.

Støttrup, J. and C. R. Sparrevohn. 2010. Stock enhancement Europe: Turbot *Psetta maxima*, pp. 219–236., in: Practical Flatfish Culture and Stock Enhancement (H. V. Daniels and W. O. Watanabe, Eds.) Ames, IA, USA: Wiley-Blackwell Publishing.

This chapter provides a thorough yet concise description of Atlantic turbot releases throughout Europe. The authors discuss the aims and rationale of turbot stocking and include information regarding marking and tagging techniques, choice of release site, release strategies, and conditioning.

Walsh, M. L., H. Fujimoto, T. Yamamoto, T. Yamada, Y. Takahashi, and Y. Yamashita. 2012. Case Studies in Flatfish Stock Enhancement: A Multi-Year Collaborative Effort to Evaluate the Impact of Acclimation Cage Conditioning for Japanese Flounder, *Paralichthys olivaceus*, in Wakasa Bay, Japan. US-Japan National Resources Panel on Aquaculture (UJNR) 39th Proceedings. Kagoshima, Japan. 35: 93-102.

The authors examined both the immediate (burying, feeding) and longer-term (movements, recapture rates) benefits of cage conditioning juvenile Japanese flounder before stocking. Recapture rates of cage-conditioned fish caught by local fishermen were greater than those of non-conditioned fish (i.e., those released directly from hatchery tanks). In addition,

burying ability was assessed by releasing recently conditioned and non-conditioned fish into tanks and quantifying the number of fish that buried. Likewise, feeding ability was assessed by providing tanks of conditioned and non-conditioned fish with prey and quantifying how many prey remained after a period. These laboratory experiments revealed that conditioned fish exhibited enhanced burying and feeding performance compared to non-conditioned fish. This work was the first to examine flatfish conditioning strategies using market data and to evaluate experimentally the performance of hatchery-reared flatfish that have been cage-conditioned.

Fairchild, E. A., N. Rennels, and W. H. Howell. 2008. Predators are attracted to acclimation cages used for winter flounder stock enhancement. Rev. Fish. Sci. 16, 262–168.

This paper addresses a negative consequence of using acclimation cages for stocking fish: that the cages in themselves may attract predators. Crab densities significantly increased in the vicinity of acclimation cages and continued to increase each day, even when cages were empty (i.e., contained no fish). Cages containing fish attracted an even higher abundance of crabs.

CHARACTERISTIC EVALUATION METHOD RELATING TO BENEDENIA DISEASE OF YELLOWTAIL (*SERIOLA QUINQUERADIATA*)

Yoshida, K

Mushiake, K., H. Yamazaki, and H. Fujimoto. 2005. Current situation of technical developments in seed production of yellowtail (*Seriola quinqueradiata*) in japan. Proceedings of the thirty-fourth U.S.-Japan aquaculture panel symposium (eds R. Stickney, R. Iwamoto, and M.Rust) : Pp1-4.

The National Center for Stock Enhancement (NCSE, formerly Japan sea-farming Association), of the Fisheries Research agency, introduced the stock enhancement program for yellowtail (*Seriola quinqueradiata* and *Seriola lalandi*) in 1977. Technical developments in induced spawning as well as larval and juvenile rearing techniques have increased the population of this species to 1 million juveniles per year at NCSE. This project faced three major drawbacks: high mortality of larvae, cannibalism, and the smaller size of released juveniles in comparison with their wild counterparts. The high mortality of larvae was overcome by utilizing strong aeration during the early larval stage, while cannibalism was controlled by grading juveniles by size selection. The two-month delay in the spawning season of reared broodstock (the usual spawning season is late April to early May), which caused the smaller size of released juveniles, was solved by developments in advanced spawning techniques. Photoperiod and water temperature manipulations were used toproduce eggs in February, thus producing yellowtail juveniles that can be released into the wild at a size similar to that of the wild stock.

Ohara, E., T. Nishimura, Y. Nagakura, T. Sakamoto, K. Mushiake, and N. Okamoto. 2005. Genetic linkage maps of two yellowtails (*Seriola quinqueradiata* and *Seriola lalandi*). *Aquaculture* 244 : Pp41-48.

The yellowtails *Seriola quinqueradiata* and *Seriola lalandi* are the most important species in marine fishery resources and aquaculture in Japan. A genetic linkage map is needed to improve efficiency of breeding by marker-assisted selection (MAS) and for the identification of commercially important genes. Therefore, we have constructed a genetic linkage map for the yellowtails using microsatellite makers. Microsatellites were isolated from agenomic DNA library of *S. quinqueradiata*. Segregation of 217 microsatellites was studied in 90 progeny from a cross between *S. quinqueradiata* and *S. lalandi*. These were used to construct separate linkage maps of a female (*S. quinqueradiata*) and a male (*S. lalandi*). Twenty-five linkage groups were distinguished in the female (*S. quinqueradiata*) map, which spanned for 473.3 centiMorgans (cM) with an average intermaker distance of 2.7 cM (total length estimated: 901.7 cM), and 21 linkage groups were distinguished in the male (*S. lalandi*) map, which spanned 584.3 cM with an average intermaker distance of 4.8 cM (total length estimated: 1715.3 cM). The microsatellite loci and genetic linkage maps will increase the efficiency of selective breeding programs for yellowtails.

Nagakura. Y., T. Yoshinaga, T. Sakamoto, K. Hattori, and N. Okamoto (2010). Susceptibility of four families derived from two Seriola species to the monogenean parasite (*Benedenia seriolae*) using a new challenge method. Journal of Fisheries Technology 3: Pp21–26 (in Japanese)

Four families of two *Seriola* species were experimentally challenged with oncomiracidia of *Benedenia seriolae*. One family was produced by crossing wild yellowtails (*S. quingueradaiata*), two families were crosses between yellowtails of the 4^{th} or 5^{th} generation passaged under laboratory conditions, and one hybrid family was a gross between yellowtail and goldstriped amberjack (*S. lalandi*). Four hundred juveniles, 100 per each of the four groups and ranging 108-111 mm in mean total length, were exposed to about 40,000 oncomitacidia of *B. seriolae* within 30 min after hatching in a 1000L tank in darkness for 4 hours. On day 10 post-challenge, the number and size of parasites were estimated on each fish. Infection intensities significantly differed in any combination of families. A significant difference was also detected in worm body length in a combination of two families. The results suggest that families of fish resistant to *B. seriolae* can be produced through breeding. The advantages of the method employed in this study for evaluating fish susceptibility against *B. seriolae* are also discussed.