



Hatchery Manual for the production of Australian Bass, Mulloway and Yellowtail Kingfish

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3. YELLOWTAIL KINGFISH (YTK)

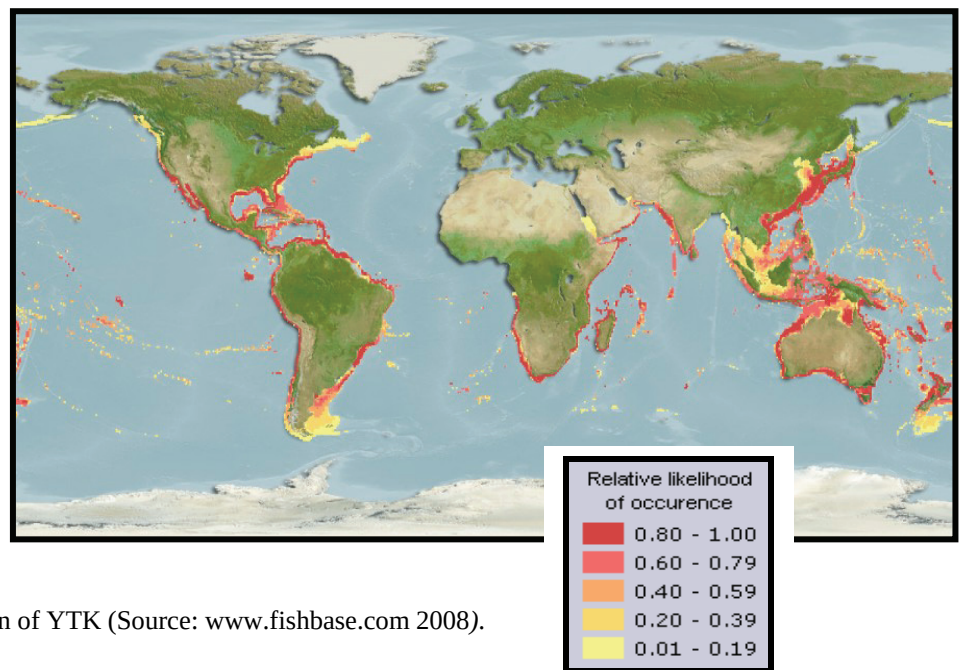
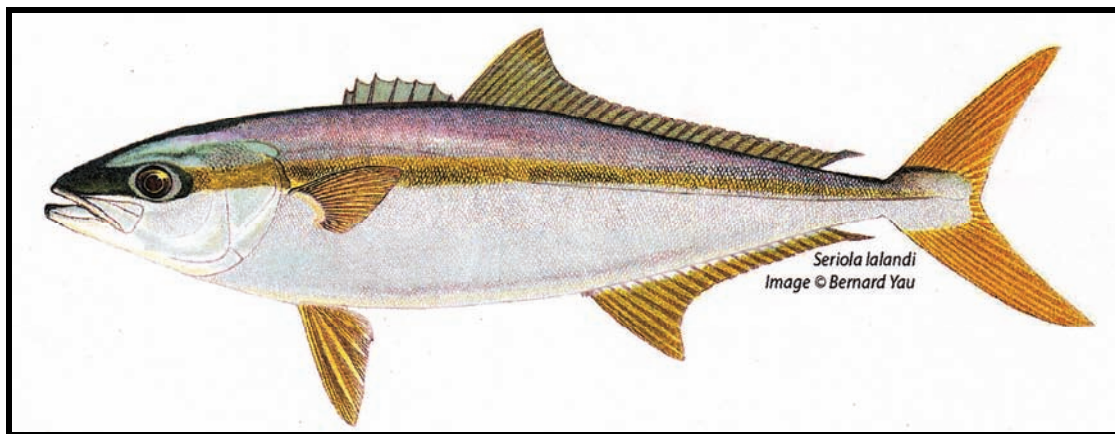


FIGURE 75: Global distribution of YTK (Source: www.fishbase.com 2008).

3.1 Appearance, Distribution and Movements

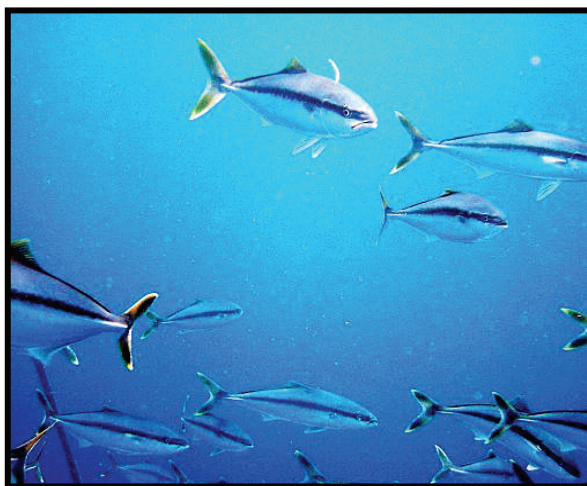
Yellowtail kingfish (*Seriola lalandi*), also known as gold striped amberjack, is a member of the family Carangidae, commonly referred to as jacks and pompanos. The body tapers posteriorly to a narrow tail stem (caudal peduncle) then broadens to a large forked yellow tail (caudal fin). The "scooped-out" centre of the tail presents a small surface area with respect to the large span resulting in little lateral displacement of water and hence low turbulence and drag. The powerful and very swift mode of swimming of YTK, involves very little movement of the head but considerable movement of the tail. The upper surface of the torpedo shaped body of YTK is generally blue or blue-green providing camouflage against the ocean depths when viewed from above. Likewise the white-silver underside provides camouflage when viewed from below against the mirror like sea surface.

Juveniles have distinctive black and bright yellow lateral bands and fins but these fade as the fish ages. By about 30 cm, YTK have assumed adult colouration.



Juvenile YTK (Source: I&I NSW website)

YTK are found circum-globally mainly in high salinity (marine) waters but prefer temperate and subtropical waters (18-24°C) (Fig. 75). Populations are disjunct occurring in the Indo-Pacific (South Africa, Walter Shoals, Amsterdam Island, Japan, Australia, New Zealand, New Caledonia, Hawaii, Rapa, Pitcairn Island, and Easter Island) and the Eastern Pacific (British Columbia, Canada to Chile. Eastern Atlantic: St. Helena, South Africa). Within Australia YTK occur from North Reef, Queensland (23°11'S) to Trigg Island, Western Australia (31°52'S), as far south as Tasmania and around Lord Howe and Norfolk island. (Australian Museum Fish-site 2008).



Schools of juveniles commonly comprising hundreds of fish up to 7 kg are generally found close to the coast. YTK occasionally enter brackish estuarine waters in pursuit of prey comprising small fish, squid and crustaceans, while larger fish are more common around deep reefs and offshore islands out to the edge of the continental shelf. Schools are commonly associated with floating debris or weed that provides a focus for gathering (Fig. 76).

FIGURE 76: School of juvenile YTK in NSW.

Note tag on uppermost fish (Source: I&I NSW Website Nov 2008)

Tagging programs have shown widespread movements of YTK. These include trans-Tasman (from Australia to New Zealand and vice versa) crossings and many large scale (>500 km) movements along the coast of New South Wales (Fig. 77).

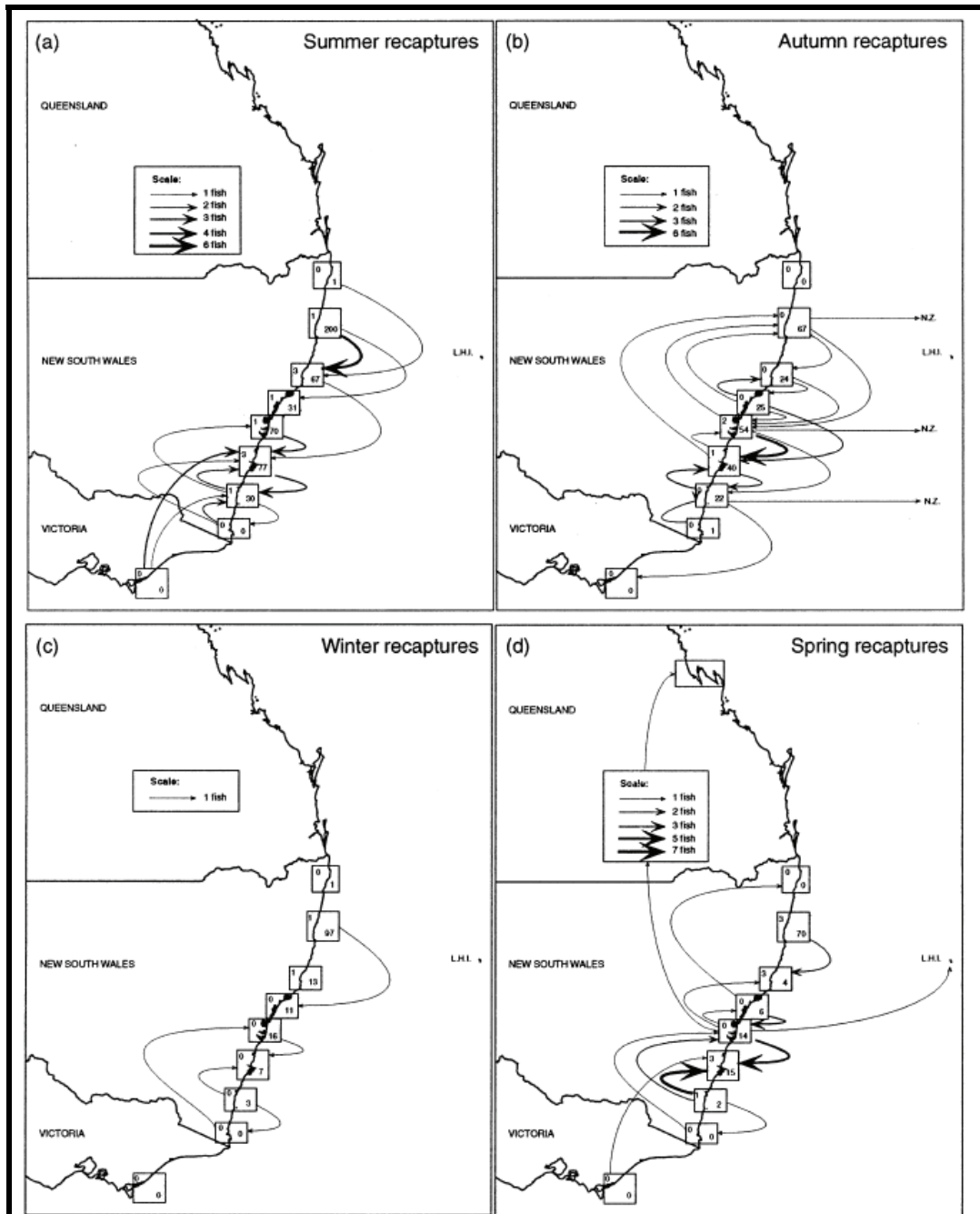


FIGURE 77: Movement of YTK by season in which they were recaptured. (Source: Gillanders et al., 2001). Only fish moving >50 km are shown. Movements shown to the right of the coast are southerly movements; those to the left represent northwards movement of fish, with the exception of one fish moving to Lord Howe Island (L.H.I.) and three fish moving to New Zealand (N.Z.). Numbers in the left corner of the boxes are fish that moved >50 km but were recaptured in the same general area as they were tagged; numbers in the right corner are fish recaptured <50 km from their release point. The size of the arrow is proportional to the number of fish.

3.2 Breeding and Early Life History

YTK are serial spawners, breeding in summer and/or autumn (Gillanders et al., 1999; Poortenaar et al., 2001) depending on seasonal sea temperature ranges of particular locations (Figs. 78 and 79). Spawning in captive YTK held under ambient temperatures and photoperiods in New Zealand by Moran et al., 2007 was consistent with that of wild fish being temperature limited to the range 17 - 24°C and occurred either just prior to dawn during the first half of the spawning season and 1 h either side of dusk in the latter half. No mass spawning events (those involving more than one female) were recorded, although two or three individual females spawned within an hour of each other on several occasions. This suggests that female YTK may spawn close together, but not necessarily at the same time.

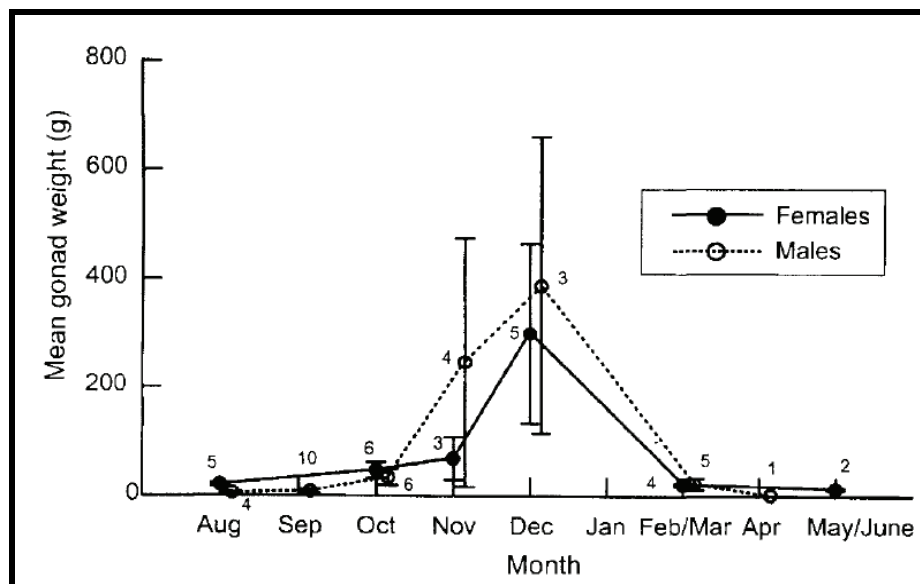


FIGURE 78: Mean gonad weight ($\pm$ SE) of female and male YTK for monthly samples from along the coast of New South Wales. (Source: Gillanders et al., 1999).

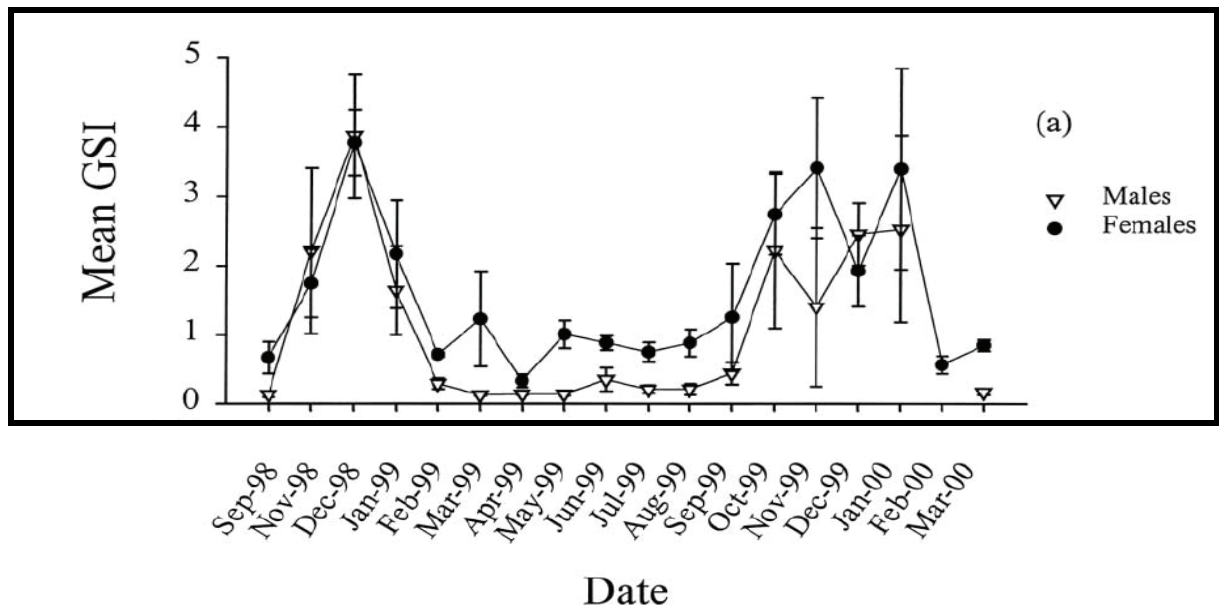


FIGURE 79: Seasonal variation in breeding condition (mean gonadal-somatic index value) of YTK in New Zealand (Source: Poortenaar et al., 2001).

The estimated mean size and age of onset of sexual maturity in YTK varies with gender and geographic location. In New South Wales (Fig. 80) the mean size and age at maturity recorded for female YTK was 834 mm and 3+ years, respectively and for males 471 mm and 0.9 years, respectively (Gillanders et al., 1999). Corresponding sizes and ages reported for YTK stocks in New Zealand (Fig. 81) are 944 mm and 7- 8 years for females and 812 mm and 4 years for males. The large differences in size and age of sexual maturity between NSW and NZ populations may be due to different growing conditions, e.g. warmer water temperatures in NSW, or behavioural and physiological differences between populations. Although no fixed genetic differences have been identified between NZ and NSW populations, large scale movements between these populations are uncommon (Poortenaar et al., 2001).

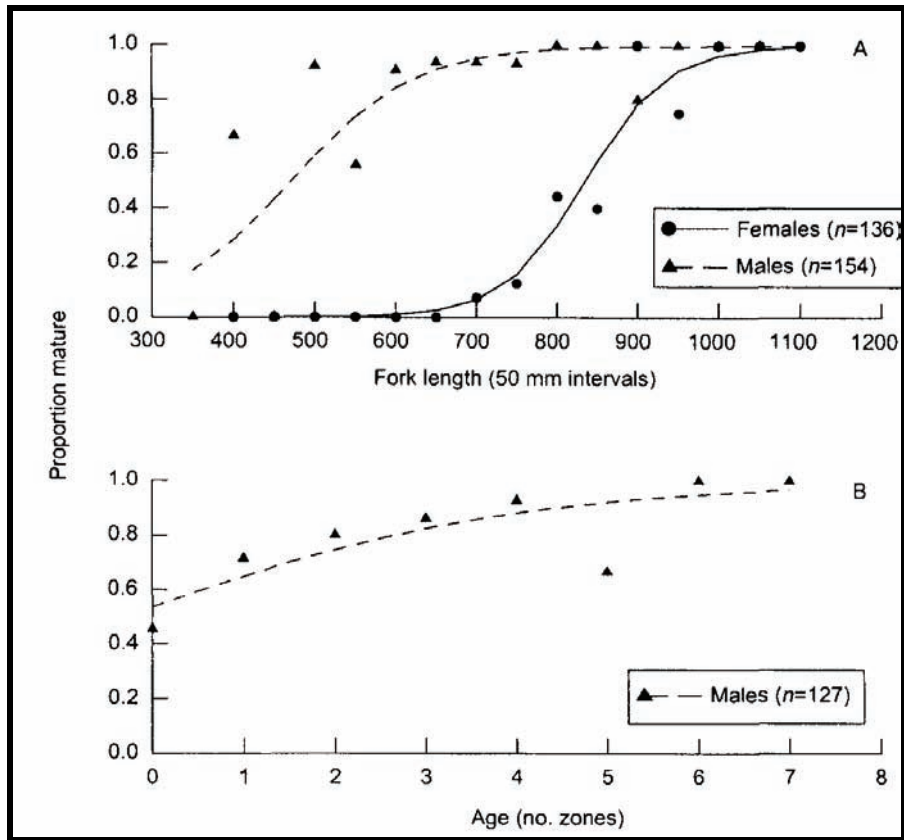


FIGURE 80: Distribution of mature *Seriola lalandi* in New South Wales: A, size; and B, age. There were insufficient age data to determine age at maturity for females. (Source: Gillanders et al., 1999).

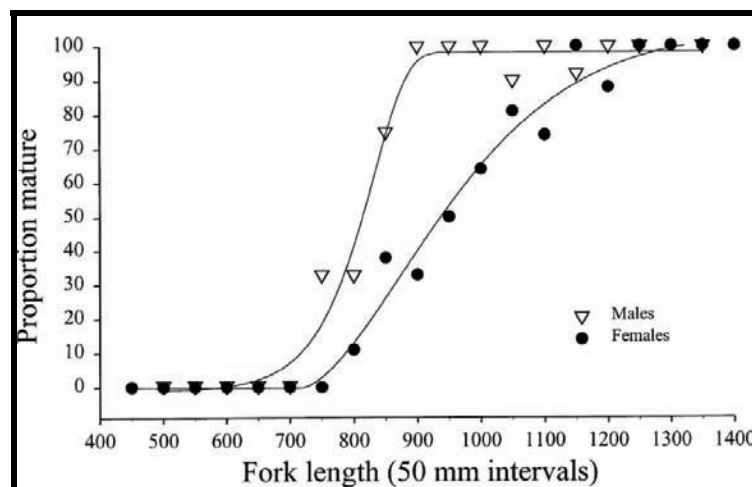


FIGURE 81: Size distribution of mature *Seriola lalandi* YTK in New Zealand. (Source: Poortenaar et al., 2001).

3.3 Food and Feeding

Predation by YTK on small schooling pelagic fish (e.g. sardines, anchovies, jack mackerel and Pacific mackerel) and cephalopods has been reported off California, Australia, New Zealand and in the Gulf of Mexico. There have been fewer studies on the diet of YTK food in the south-western Atlantic, with reports the diet comprises juvenile Argentine anchovy (81%), jack mackerel (7%) and chub mackerel (1.9%). In Argentina, YTK tend to be restricted to rocky reefs, which have a scarce and patchy distribution in the region, and this affinity to reef habitats may be related to their feeding behaviour. Observations on the feeding tactics of YTK in the Gulf of California showed a high degree of cooperation among individuals while performing well-coordinated foraging behaviour that involved fish enclosing schooling prey against the rocky reefs prior to feeding, (Vergani et al., 2008).

3.4 Growth, Longevity and Mortality

YTK have a maximum recorded length, weight and age of 250 cm, 96.8 kg and 21 years, respectively. In Australia, the largest recorded YTK was about 200 cm in length and 70 kg in weight but as indicated by size and age frequency catch data for NSW (Figs. 82, 83 and 84), fish considered large are commonly around 100 cm and 10-15 kg and 10-12 years old.



FIGURE 82: Typical haul of YTK Photograph: Paul Jennings, (Source: <http://www.sardi.sa.gov.au>)

NSW stocks of YTK grow to a mean length of about 450 mm in their first year. Thereafter to an age of about 11 years annual growth increments are essentially constant, progressively diminishing to about 90 mm in year 6. Growth of NSW stocks of YTK under relatively warm temperature regimes is considerably faster but terminal size considerably smaller than counterparts in colder New Zealand and the United States waters. Annual growth rates of 144 mm/y for 500 mm TL fish in NSW compare with 98 mm/y in New Zealand and a range of 34 -10 mm/y in the USA for similar size fish (Gillanders et al.,1999; Stewart et al., 2004).

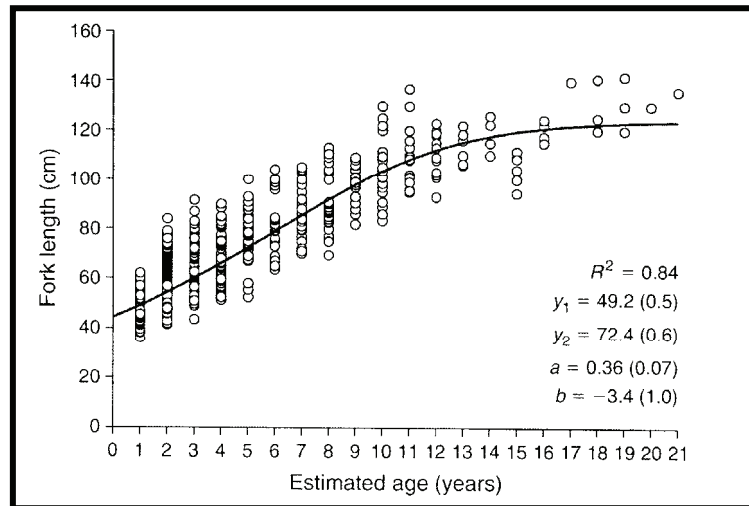


FIGURE 83: General growth curve for YTK off New South Wales (Source: Stewart et al., 2004).

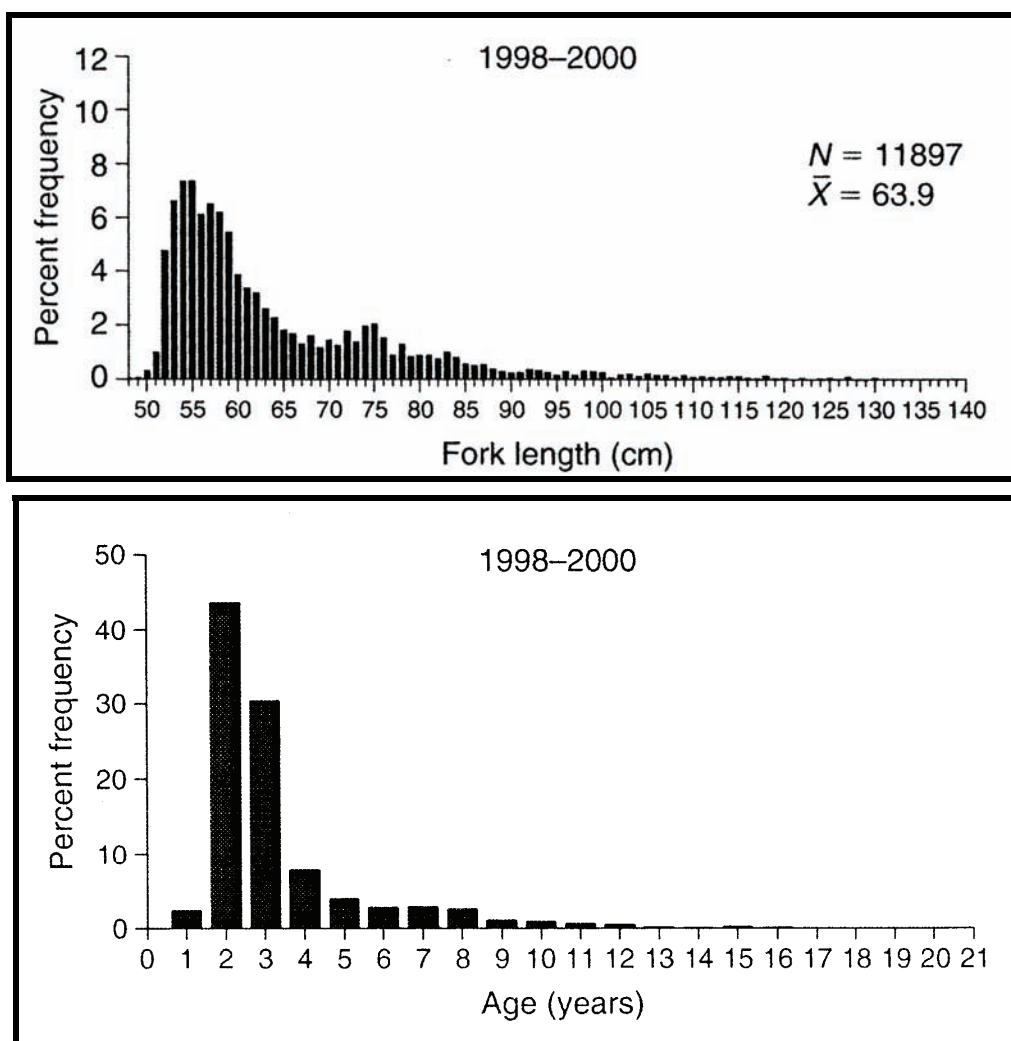


FIGURE 84: Size and estimated age composition of commercial landings of YTK between 1998 and 2000. (Source: Stewart et al., 2004).

Combined natural and fishing mortality rates of YTK on the east coast of Australia (NSW) has been estimated in the range from 35 to 55% per year for 3-14 year old fish with the major losses being due to fishing rather than natural (predation and disease) factors.(Stewart et al., 2004)

3.5 Hatchery Protocols - Yellowtail Kingfish (YTK) -

3.5.1 Introduction

For the most part broodstock, larval and nursery rearing equipment and operating protocols already described for mullet in this document (see Chapter 2), and for snapper in good detail by Partridge et al., 2003 also apply to YTK. What follows is an account of variations on equipment and operating protocols that apply generally to kingfish (*Seriola* spp.) and in particular to YTK (*Seriola lalandi lalandi*) as described in Moran (2007), Poortenaar et al., (2001) and PIRSA (2002).

3.5.2 Husbandry of captive broodstock

As with many other fish including Australian Bass and mullet (see Chapters 1 and 2) viable gametes can be obtained from either wild, captive or cultured YTK broodstock. However wild, YTK broodstock suffer severe stress at capture and offshore spawning sites are relatively inaccessible. Breeding condition regresses while the fish are held in interim quarantine holding facilities. Quarantined fish are typically held for a fortnight before being transferred to breeding tanks. During this period they are rid of parasites by bathing in specialised chemo-therapeutic or formalin baths followed by a freshwater bath and, ideally, tested for carrier status in relation to VNN (see Chapter 4), although no reliable, non-destructive test is available as yet for nodavirus detection.

Breeding tanks are typically 20-70 m³ or occasionally larger, and at least 2 m deep with reported stocking rates of 5-14kg/m³. Although captive broodstock are usually fed fresh or frozen premium quality diets e.g. chopped fish, squid, it is recommended that they are weaned onto a semi-moist or dry pellet (50.5% protein, 24% lipid) with vitamin and mineral supplements. Reason for the latter is that in kingfish (*Seriola* species), a diet of soft-dry pellets may produce 2-5 times more eggs and larvae of superior quality (higher fertilization rates and fingerling yields). While the majority of research on broodstock nutrition in marine fish concerns the levels of essential fatty acids (EFA's) required to support normal larval development, the optimal amounts of various EFA's for *Seriola* broodstock are not known. It does however appear that the ratio of EFA is likely to be more important than the absolute quantities of the individual EFA's. Several studies on the broodstock nutrition of congeners of YTK (*S. quinqueradiata*) have shown that certain carotenoids, in particular astaxanthin, improves egg and larval quality. In one study, astaxanthin improved egg buoyancy, fertilization and hatching rate and prolonged the period of egg production. However not all carotenoids appear beneficial to broodstock. For example β carotene is very poorly absorbed and high levels of β carotene in broodstock diets have little effect on the subsequent egg and larval quality. Feeds are administered at 1 to 3 % or 10 % of total fish weight daily for pellet and wet diets, respectively.

Wild caught YTK broodstock spawn spontaneously in captivity within 1 to 2 breeding seasons of domestication. If in the shorter term, hormone treatments are required to stimulate maturation and spawning, the timing of treatments need to coincide with critical stages of reproductive development. Females with oocytes less than 650 μ m in diameter do not spawn, females with oocytes around 700 μ m spawn but subsequent fertilization and hatch rates are poor, whereas females with oocytes greater than 800 μ m in diameter produced vast quantities of good quality eggs. As with Australian Bass (Chapter 1) and mullet (Chapter 2), samples of oocytes can be collected from anaesthetised broodstock by inserting a biopsy tube (catheter) into the genital opening. Three different hormonal treatments have been successfully used to induce oocyte maturation and ovulation in kingfish, including a single injection of hCG at 500 IU/kg (as described for Australian Bass (see Chapter, 1 pp 12 to 18), priming injections of HCG one day after the single hCG, and injection and single implantation of LHRHa at 220-400 μ g/kg in a cholesterol pellet. While the latter (LHRH implantation) is superior in terms of egg quality and yields of eggs, a single injection of hCG is a much cheaper procedure.

As discussed above (Chapter 3.2), YTK are serial spawners, breeding under ambient conditions beginning in late spring or early summer and continuing to autumn over temperatures in the range 17-24°C. Photoperiod at the start of the natural spawning season (November) is about 13.5 h light :10.5 h dark, and increases to a maximum day length in mid December (14 h light:10 h dark) before decreasing to 11.5 h light:12.5 h dark at the end of the spawning season (April). In the southern hemisphere, when held under constant temperature 20 $\pm$ 1°C and natural photoperiods, YTK spawning and larvi-culture occurs from November until the end of February, after which time egg production and quality tends to decrease to a degree that makes it unviable for commercial purposes. At PSFI, year-round, on-demand spawning of captive YTK broodfish has been achieved from a single tank of fish (see Chapter 2 for description of tanks) containing 7 pairs of wild collected fish. After initial exposure to a truncated phototherm (Table 8), fish have

been held at a constant 10h:14h light: dark regime and at 16°C. Spontaneous spawning can be induced to occur 3-4 days after the water temperature is increased from 16 to 22°C within 24-48h.

Courtship behaviour involves one male and female, and consists of a high-speed pursuit punctuated by stalling, nipping and touching. This lasts for approximately 0.5–1.5 h until, immediately prior to spawning, males nip at the female gonoduct, presumably to induce spawning. An additional male becomes involved at this stage in 50% of spawns. Release of gametes involves frenzied circling behaviour near the bottom of the tank and lasts 20-25 seconds.

Spawning occurs either just prior to dawn during the first half of the spawning season and 1 h either side of dusk in the latter half. In commercial hatchery operations it is often desirable to extend the natural spawning season or stimulate out-of-season spawning to increase production. This is achieved with YTK as in mullet (see Chapter 2 for detailed description of equipment and protocols) by controlling the temperature and photoperiod into abbreviated seasonal cycles called photo-therms. Optimum spawning temperature for YTK is 21.5°C. As discussed above, at the start of the breeding season spawning occurs early in the morning around dawn or the time of the simulated sunrise in controlled photo-therm systems, meaning eggs could be collected very close to the spawning event. However after December spawning tends to occur at night, precluding the immediate collection of eggs.

TABLE 8: Compressed seasonal photoperiod and temperature regime used in the phototherm rooms at PSFI. This regimen is suitable for mullet and yellowtail kingfish as well as snapper, for which it was originally designed.

Compressed (15 min) regime						
Date	Daylength	Light ON	Light OFF	CHANGED	Temperature °C	Day
19-Dec	10.5	6 45	17 15		16	1
24-Dec	11.25	6 15	17 30		16.5	5
29-Dec	11.75	6 00	17 45		16.5	10
3-Jan	12.5	5 30	18 00		16.7	15
8-Jan	13	5 15	18 15		16.95	20
13-Jan	13.5	5 00	18 30		17.2	25
18-Jan	13.5	5 00	18 30		17.65	30
23-Jan	14.25	4 45	19 00		18.1	35
28-Jan	14.25	4 45	19 00		18.95	40
2-Feb	14.25	4 45	19 00		19.8	45
7-Feb	14.25	4 45	19 00		20.4	50
12-Feb	13.25	5 15	18 30		21	55
17-Feb	12.75	5 30	18 15		21.4	60
22-Feb	12.25	5 45	18 00		21.8	65
27-Feb	11.75	6 00	17 45		22	70
4-Mar	11.25	6 15	17 30		22.2	75
9-Mar	10.75	6 30	17 15		21.85	80
14-Mar	10.25	6 45	17 00		21.5	85
19-Mar	10.25	6 45	17 00		20.5	90
24-Mar	10	7 00	17 00		19.5	95
29-Mar	10	7 00	17 00		18.85	100
3-Apr	10	7 00	17 00		18.2	105
8-Apr	10	7 00	17 00		16	110
13-Apr	10	7 00	17 00		16	115
18-Apr	10.5	6 45	17 15		16	120

3.6 Larviculture (Based on PIRSA, 2002; Moran et al., 2007; Benetti et al., 2002; Carton 2005; Kolkovski, 2005 and Kolkovski and Sakakura, 2004.)

3.6.1 Introduction and background

Spawned eggs from well nurtured YTK broodstock are positively buoyant, have a high fertilisation rate (90- 99%), range from 1.33 to 1.50 mm in diameter and have a single oil droplet 0.30–0.33 mm in diameter. In the case of natural spawning, fertilized eggs are collected from the surface of broodstock tanks with nets or screens or using automatic skimmers as described for mullockway hatchery production (see Chapter 2). Collected eggs are rinsed and treated with 100 ppm formalin or preferably with ozone to disinfect them of bacteria, fungi and viruses. Disinfected fertilised eggs are placed in sloping bottom tanks and maintained under 12:12 photoperiod conditions. As illustrated in Figure 85, incubation (time to hatch) is 2-4 days depending on water temperature in the range 16 and 24°C. Mean $\pm$ s.d. egg viability within the floating fraction over a complete spring / summer reproductive season has been reported as 74% $\pm$ 17% (approximate range 50-90%).

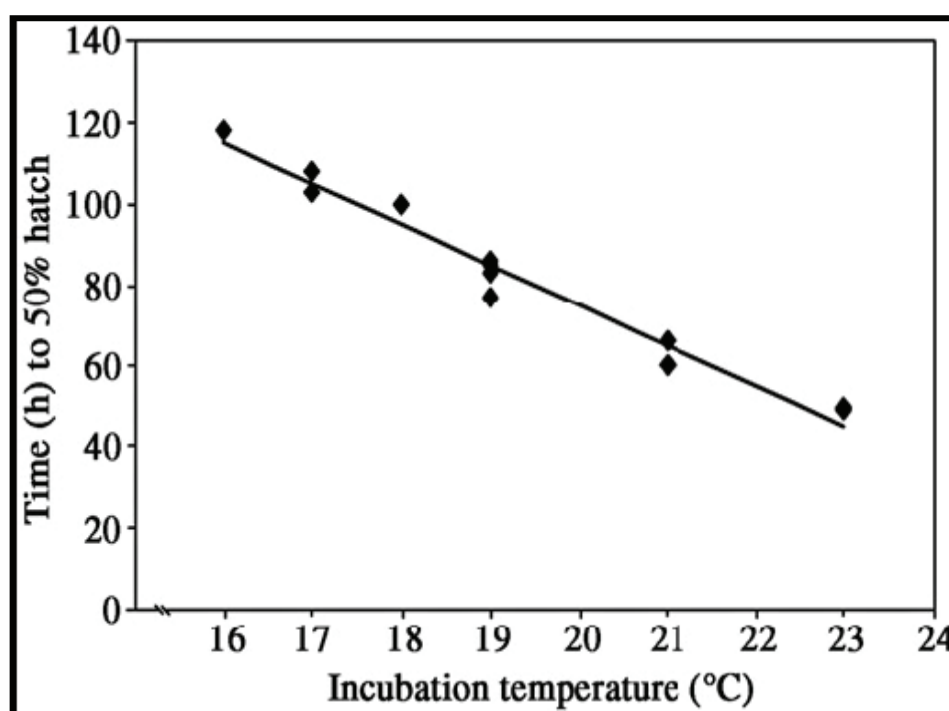


FIGURE 85: Correlation between incubation temperature and time to 50% hatch for YTK . The regression function is represented by $y = -9.99x + 274.84$, $r = -0.98$, $P \leq 0.01$. (Source Moran et al., 2007). Correlation between incubation temperature and time to 50% hatch for YTK. The regression function is represented by $y = -9.99x + 274.84$, $r = -0.98$, $P \leq 0.01$. (Source Moran et al., 2007).

Indistinct cell margins and asymmetrical cleavage during blastomere formation (Fig. 86g) are the most common deformities observed. Egg and oil droplet volume may decrease by 15–20% over the spawning season, though no associated fall in egg and larval viability has been reported. For instance while larvae hatch at a smaller length with a larger yolk sac and oil droplet at warmer incubation temperatures, there is no substantial difference in the maximum larval length reached at the onset of first feeding across incubation temperatures in the range 16 to 24°C.

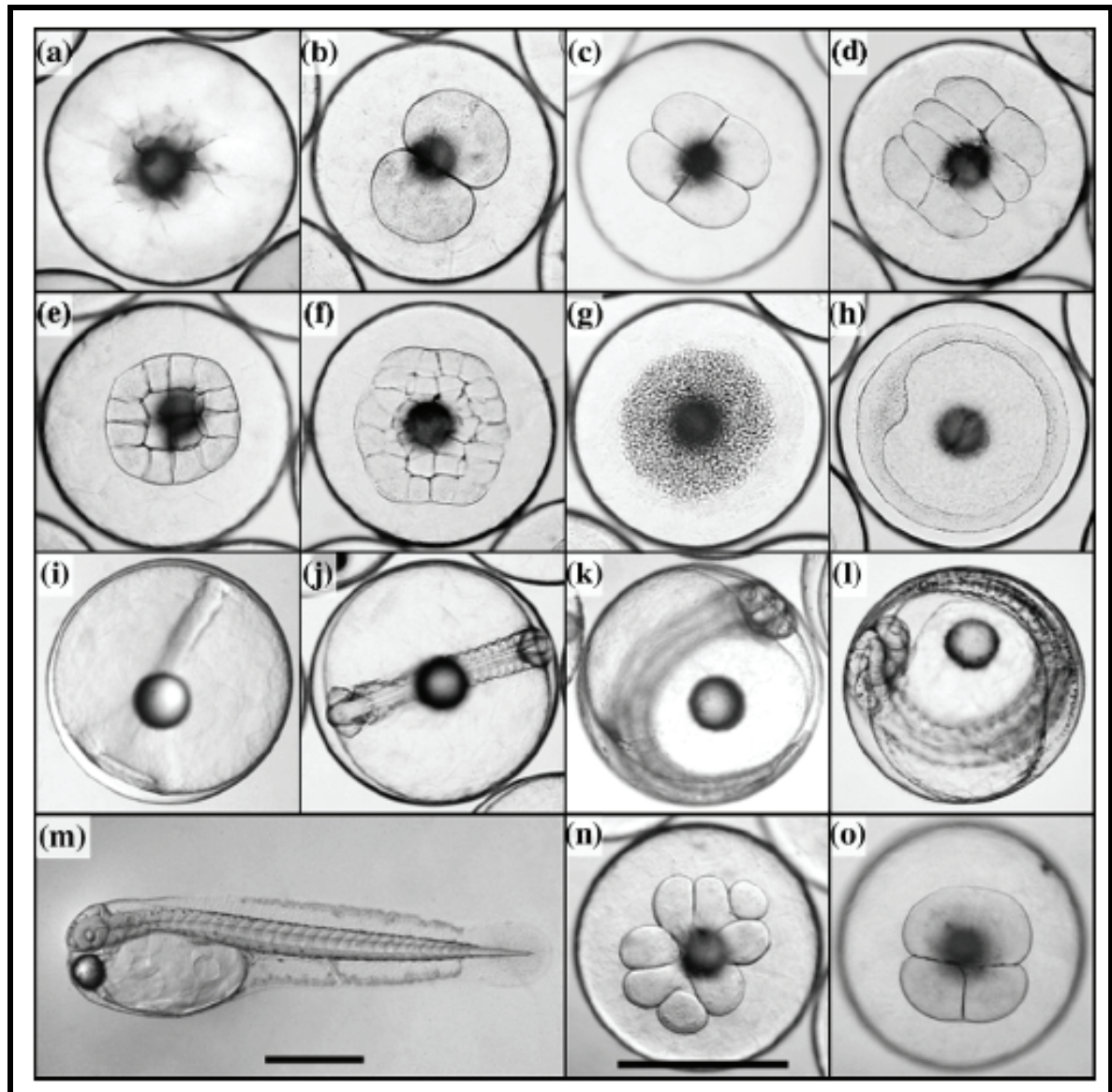


Figure 86: Developmental stages and cleavage abnormalities of YTK: (a) pre-cleavage; (b) 2 cell; (c) 4 cell; (d) 8 cell; (e) 16 cell; (f) 32 cell; (g) mid-stage blastula; (h) gastrula; (i) appearance of embryo; (j) 20 myomere embryo; (k) advanced embryo; (l) pre-hatch embryo; (m) larva 4 h posthatch; (n) asymmetrical cleavage in blastula; (o) indistinct cell margins in blastula. Scale bar for (a)–(l) and (n)–(o) shown in (n); scale bars represent 1 mm. (Source: Moran et al 2007).

3.6.2 Larviculture protocols

YTK (*Seriola* spp) larvae grow faster than many other marine finfish including those of Australian Bass (see Chapter 1) and mullet (see Chapter 2) (Figs. 87, 88 and 89). The eggs and first feeding (Day 3-4) larvae are relatively large averaging about 1.1 mm diameter and 4.5 mm TL, respectively. Therefore relatively standard hatchery equipment and rearing protocols as already described in detail for mullet (see Chapters 2) can be followed (Table 7). YTK larvae are stocked into rearing vessels commonly in the range 1 to 5 m³ as previously described for mullet (see Chapter 2) at densities ranging from 20-100/l into water conditioned with algae, and aeration is used to maintain the larvae in gentle rolling suspension to help reduce the incidence of deformities and early mortalities (see Chapter 4 below). As for mullet larvae (see Chapter 2), surface skimmers fitted to larval rearing tanks are essential to ensure normal swim bladder inflation. YTK larvae begin feeding 3-4 days after hatch once the yolk-sac has been absorbed and jaw development completed.

Feeding performance of YTK larvae increases with age and light intensity under both clearwater and greenwater rearing conditions, demonstrating that visual feeding proficiency increases with larval stage. Feeding intensity remains low over the first 3 days of feeding regardless of light intensity. On days 6 and 7 after hatch, larvae show considerably higher feeding intensity particularly at light intensities in the range 8 and 17 $\mu\text{mol}/\text{sec}/\text{m}^2$ (≈ 1600 to 3400 lux). This improvement indicates an ontogenetic shift in sensory acuity and/or swimming competence. First-feeding larvae perform equally well in clear-water and green-water up to algal cell densities of 8×10^4 /mL, although at a low light intensity of $0.1 \mu\text{mol}/\text{sec}/\text{m}^2$ (≈ 20 lux), feeding performance is significantly constrained. The ability of YTK larvae to capture and consume free-swimming prey during the first-feeding window is also impeded under high algae cell densities above 16×10^4 cells/mL, thereby undermining the suitability for large scale production in outdoor greenwater ponds.

Hatchery reared YTK larvae are initially fed enriched small or large strain rotifers at 10-30 rotifers/mL and, if available, wild collected or pond produced copepods. Enriched *Artemia* metanauplii are subsequently added to the diet 10-14 days after hatch. Feeding protocols are in fact essentially identical to those already described in detail for mullet (see Chapters 2.8.1 and 2.8.2 for details). Metamorphosis occurs approximately 20 days after hatching coincident with weaning onto inert formulated foods and is usually completed 40-50 days after hatching.

TABLE 9: Optimal rearing parameters and feeding schedule for YTK larvae used at PSFI.

Species: Yellowtail Kingfish (<i>Seriola lalandi</i>)			
Parameter	Target	dah	Adjustment
pH	7.6 - 8.2	0+	
Dissolved Oxygen (mg/l)	>6.00	0+	Use compressed oxygen diffuser to maintain saturation level
Temperature (°C)	22	0+	Increase post SB inflation
Salinity (ppt)	25 - 35	0+	
Water Exchange (%/day)	100 - 200	0+	Increase exchange as larvae develop
Surface Skimmer (hrs/day)	24	2+	Monitor skimmer to ensure larvae at water surface are not affected
Photoperiod (L:D)	(12:12) (18:06)	(0+) (6+)	Increase post SB inflation
Light Intensity (Lux)	225-400	0+	
Green-water (cells/ml)	1.4 x 10 ⁶	0+	Pro-Aqua* concentrate 57x10 ⁹ per ml
Rotifer (R/ml)	20.0 - 5.0	4+	Initial 20/mL until feeding and then increase frequency of reduced concentration (e.g. 4x5/mL/d).
Artemia (A/ml)	0.2 - 2.0	12+	0.2/mL until weaned, then increase concentration and frequency
Weaning Diet size (µm)	200 - 800	18+	Commence weaning at 10 mm TL

*Algae concentrate used Rotifer Diet-3600 (*Nannochloropsis/Tetraselmis* blend) from Reed Mariculture Instant Algae, imported via Proaqua Australia. <http://www.proaqua.net.au>

NB. One lux is equal to 1.46 milliwatts (0.00146 watts) Full daylight at noon ≈100,000 lux ≈ 10,000 foot candle ≈ 500 µmol/m²/sec (microeinsteins/square metre/second)

Water temperature is typically maintained at the higher end of the optimum range (20–28 °C), although recent data suggest that 21-23°C may be optimal for survival and low incidence of deformities (pers. comm., SARDI, 2010). The water exchange rate is gradually increased from 4 L/minute (1 – 2 x exchanges per day) at the time of stocking, up to 20 L/minute (4 - 8x exchanges per day) immediately prior to weaning.

As in other finfish larvae, such as those of Australian Bass (Chapter 1) and mullet (see Chapter 2), first feeding in YTK larvae is a major hurdle and adequate nutrition is critical to the success of this phase. Also in common with larvae of most other marine finfish, essential fatty acids (EFA's), in particular docosahexaenoic acid (DHA), are critical for normal development. DHA is accumulated in the central nervous system of YTK larvae and is essential not only for activity and vigour but also for the development of schooling behaviour in juveniles. Studies on the effect of the different EFA's on the growth and survival of kingfish larvae such as those of *S. quinquerradiata* have shown that growth and survival rate of those fed DHA-enriched *Artemia* at 2.1- 2.5% dry wt./day is up to ten times better (88%) than larvae fed *Artemia* enriched with other EFA s including eicosapentaenoic acid (EPA), arachidonic acid (AA) or oleic acid (OA).

Generally there are 2 peaks of mortality in rearing kingfish (*Seriola* spp) larvae. The first is the so-called 'critical period' of high mortality from hatch to first feeding especially during the mouth-opening phase/first feeding stage (day 3 or 4 after hatch), during which larvae sink to the bottom of rearing tanks. This phenomenon can be mitigated by imposing strong upwelling currents that are also of substantial benefit for retaining the inert particles longer in the water column especially if live feeds are partially or totally substituted by inert micro particulate diets. The second mortality peak is caused by cannibalism. The first obvious signs of aggressive interactions becomes evident as early as 12 days after hatch (6-7 mm total length), with both the large and medium size individuals displaying threatening (aiming) behaviour at smaller siblings. The onset of cannibalism does not occur in small larvae but coincides with increased growth rate and size heterogeneity and the onset of metamorphosis 18 to 22 days after hatch at a mean size of 10 mm, cannibals being able to successfully prey on fish up to half of their own body size.

Fortunately cannibalism is a relatively fleeting phenomenon in YTK progressively waning from around day 30 after hatch (>12mm TL) as schooling behaviour takes over. A clear dominance hierarchy exists within schools of post-larvae although it is likely that the ranking order changes with time. The aggressive behaviour of larvae is affected by temperature and light intensity, stocking density, feeding level, starvation, and the size-difference between fish (heterogeneity). Aggression in general, and cannibalism in particular, is exacerbated by increasing temperature over the range 15–30°C, starvation (food deprivation) periods exceeding 12 hours and is highest at medium light intensity. Numbers of aggressive encounters decreases as density is increased probably due to the inability of predators to single out and attack individual smaller prey at very high densities. Aggression in dominant fish also increases at higher densities and based on practical experience with other highly aggressive carnivorous fish species, it is likely that the overall level of cannibalism at intermediate densities will increase with increasing density. Although the above factors exaggerate the level of aggression in larvae, aggression still persists under low density, well-fed conditions amongst individuals of the same size. In order to reduce the level of cannibalism in cultured conditions the recommended optimum stocking density is 3 fish /L. Fish should also be size-graded regularly (e.g. weekly) beginning at about 10 mm TL (Fig. 90). At night time, juveniles cease swimming and drift at the surface in dense aggregations and are therefore easy to handle and grade. Indeed night time grading, has shown to increase survival yields by 1.5 to 3 times and is therefore recommended .

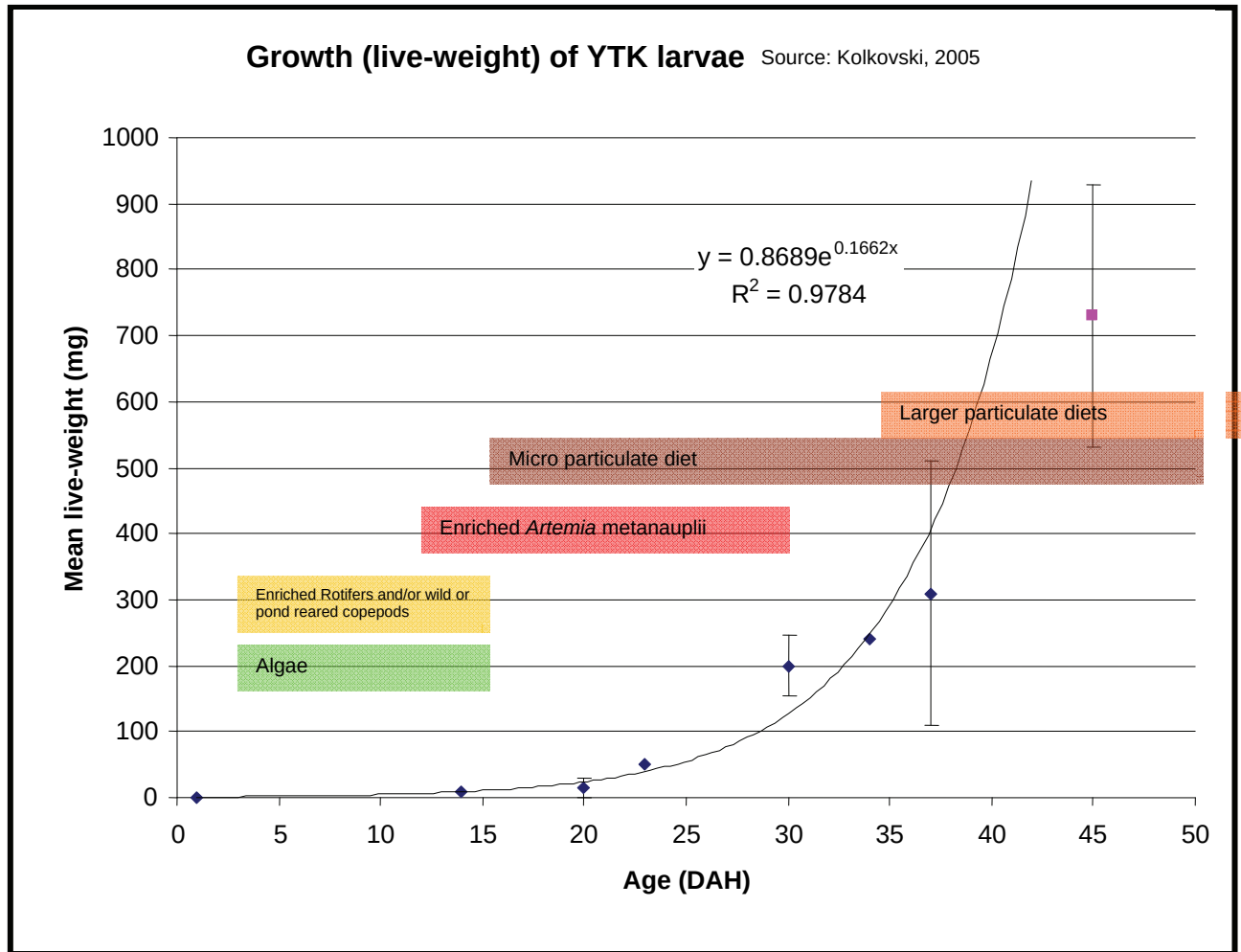


FIGURE 87: Typical exponential growth curve and feeding regimen for YTK larvae and post-larvae. (Source: redrawn from Kolkovski, 2005).

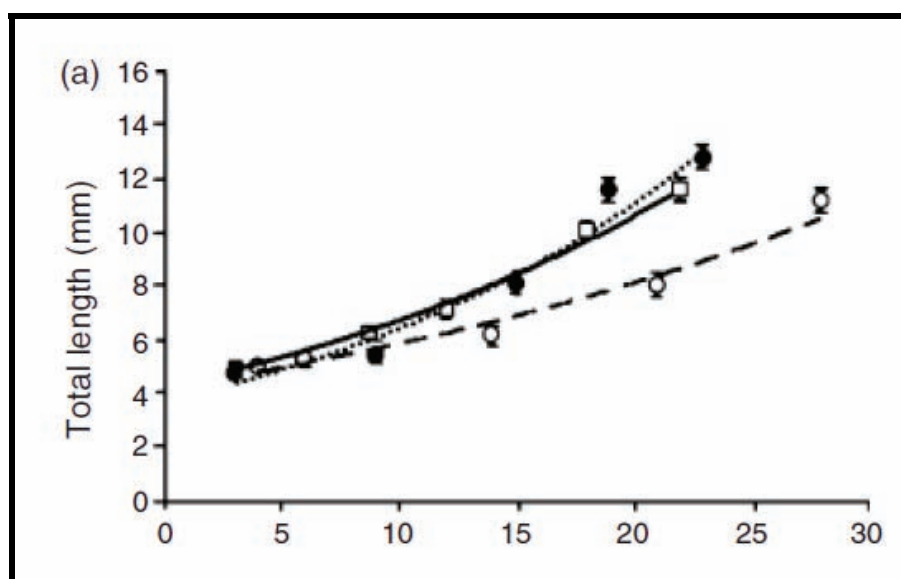


FIGURE 88: Typical exponential growth curves for three commercial batches of YTK larvae and post larvae cultured between 21 and 23 °C. (a) Total body length during development (mean $\pm$ SE), $n = 20$ for each data point): Batch 1 ($\square$) $y = 3.68 e^{(0.055x)}$, $r = 0.99$; Batch 2 ($\circ$) $y = 4.18 e^{(0.033x)}$, $r = 1.00$; Batch 3 ($\bullet$) $y = 54.22 e^{(0.048x)}$, $r = 0.99$, (Source: Moran et al., 2007).

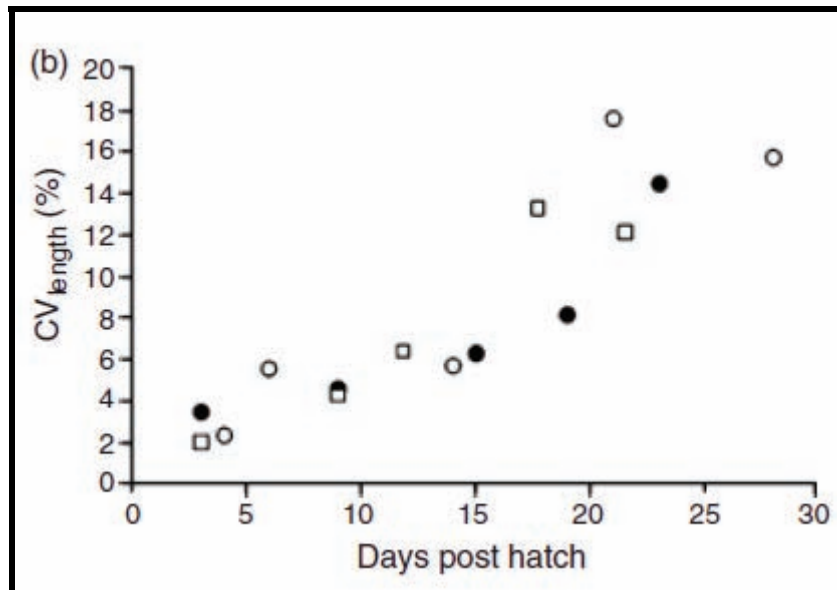


FIGURE 89: Co-efficient of variation of body length during development of YTK larvae Symbols as in Figure 90. (Source: Moran et al., 2007).

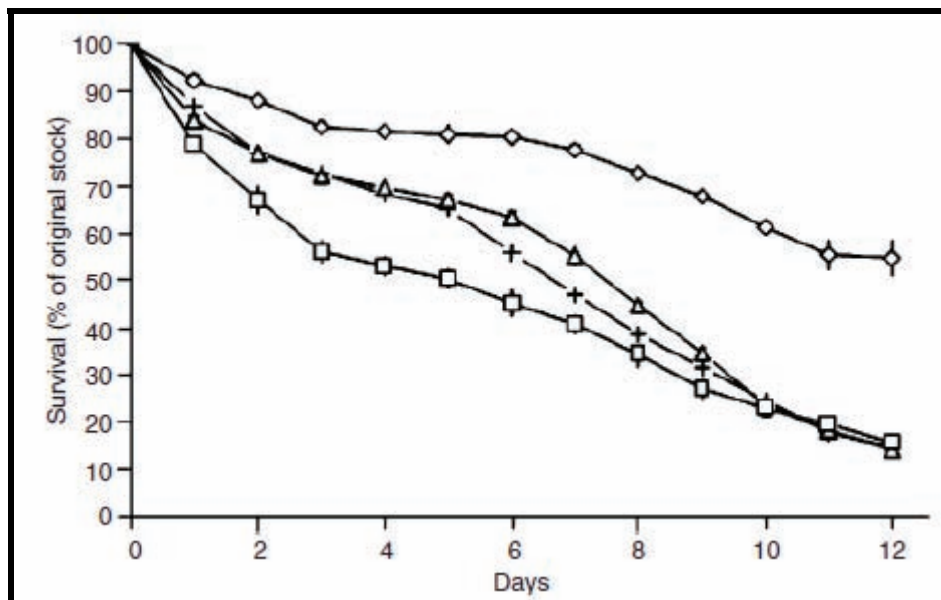


FIGURE 90: Larval survival during the grading trial. Survival is shown as the mean $\pm$ SE survival from three replicate tanks (+) = control ungraded; (◇) = large grade; (Δ) = medium grade; (□) = small grade. (Source: Moran et al., 2007).

While relatively low rates of survival are to be expected with YTK larval as with those of other species of kingfish, survival rates to metamorphosis and weaning have nevertheless been progressively improved from initial rates of 0.1 to 2 % up to 5 to 10% currently with adoption of the measures discussed above.

An important lingering problem with hatchery production of YTK and other kingfish is the level of deformities. This problem is common to fish cultured in different places including Japan, Australia and New Zealand. These deformities range from fused vertebrae and scoliosis, bent and/or shortened lower jaws, incomplete or absent gill covers (opercula) and compacted body and tails (Figure 91a-d). Although the full array of factors causing deformities are yet to be determined and negated, significant progress in mitigating these deformities has been made through improved nutrition. Feeding *Artemia metanauplii* (but not rotifers) enriched with 'mega' doses of vitamins E and C to YTK larvae is effective in significantly lowering the incidence of these deformities.

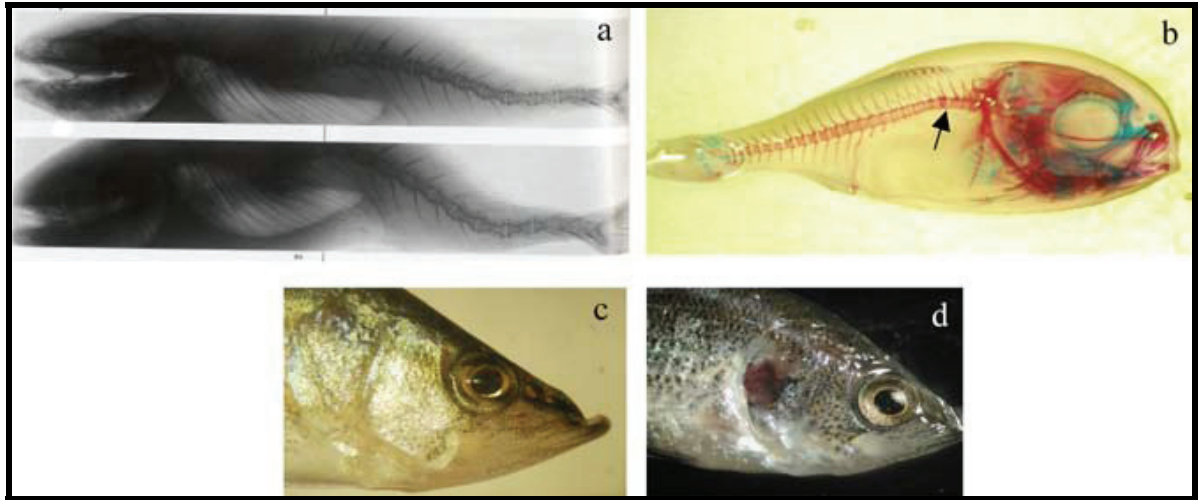


FIGURE 91: Photomicrographs of common deformities of hatchery-reared YTK. (Source: Kolkovski, 2005).

3.6.3 Weaning

Recent dietary research on greater amberjack (*Seriola dumerilli*) showed that there may be considerable advantages to growth performance and body condition factor in applying the following dietary protocols to nursery rearing and subsequent on farming of YTK (Fig. 92):

- Feed juveniles from the point of weaning through to a weight in the range 300 to 400g on soft pellets comprising a 50:50 blend of fish and formulated pellets (35 % moisture)
- change the diet to a 40% moisture soft pellet for a short interim period of 1 month
- change the diet again to an intermediate 20% moisture soft pellet for the next 3 months
- Adopt a standard dry diet (7% moisture) thereafter.

3.7 Summary of “best-practice” Rearing Regimes for YTK

“Best-practice” rearing regimes for YTK larvae at PSFI is summarized in Table 10.

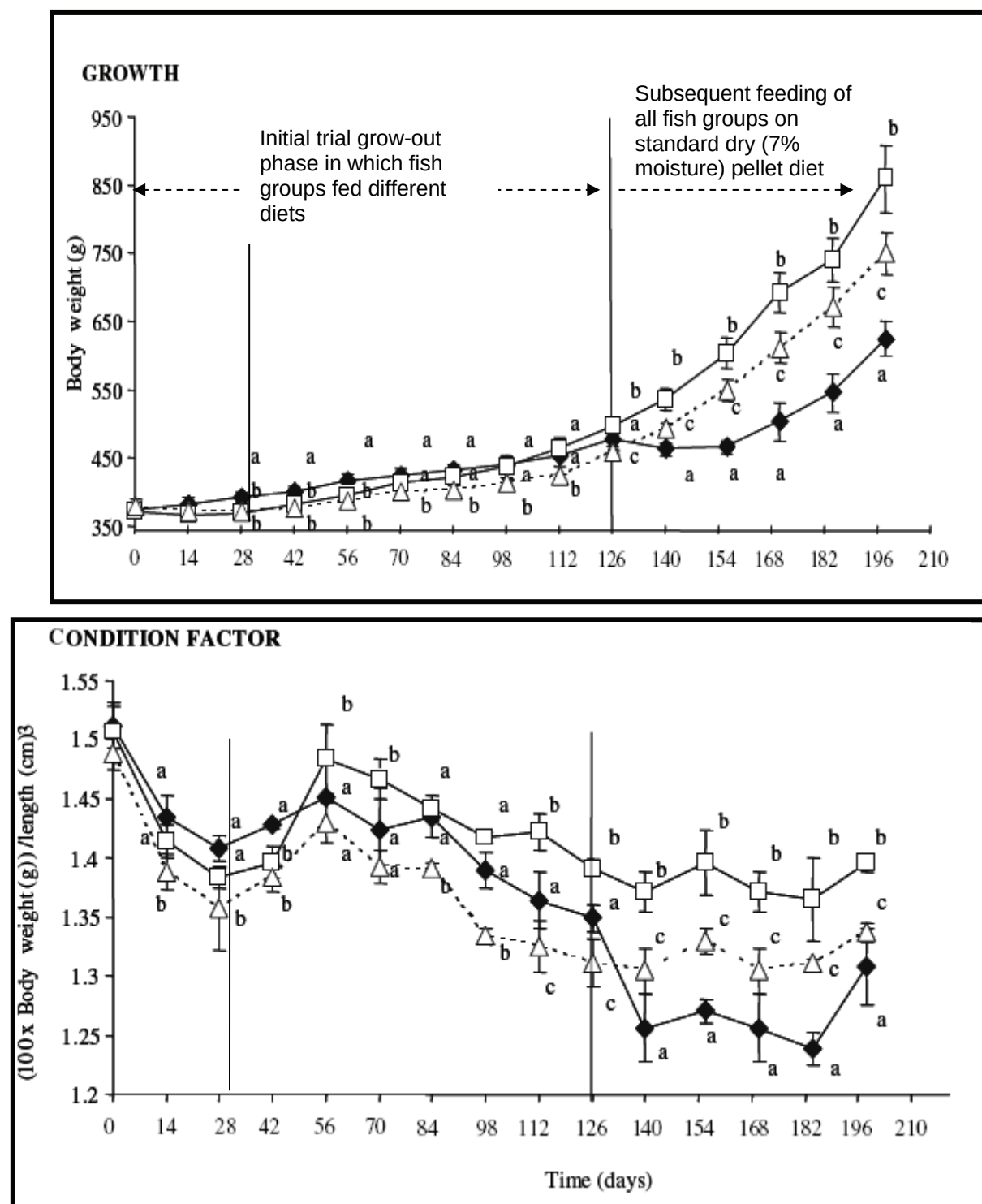


FIGURE 92: Effect of formulated diet moisture content (♦ 40%, □ 20%, Δ 7%) on: (a) growth and (b), condition factor of juvenile amberjack (*Seriola dumerilli*). Values are mean ± SD (of three replicate groups). Statistical differences within sample days are represented by different letters (a, b, c). All fish fed moist diet comprising a blend of 50% fish flesh and 50% dry crushed pellet from weaning to commencement of experiment (Source: Papadakis et al., 2008).

TABLE 10: The “best-practice” regime for yellowtail kingfish larval rearing used at PSFI.

SPECIES: YELLOWTAIL KINGFISH (<i>SERIOLA LALANDI</i>)			
BREEDING & DEVELOPMENT	UNIT		COMMENT
BROODSTOCK ORIGIN	WILD-CAUGHT		CAPTURED FROM INSHORE COASTAL AND DEEP OFFSHORE OCEAN SITES
BROODSTOCK TANK SIZE	22,000 L		
SPAWNING INDUCTION	PHOTOTHERM		INCREASE WATER TEMPERATURE FROM 16 TO 22°C OVER 24 -48 H INDUCES SPAWNING
TANK SIZE FOR SPAWNING	22,000 L		1 : 1 MALE:FEMALE (14 FISH)
LATENCY PERIOD TO SPAWNING	72 – 96 H		AFTER TEMPERATURE REACHES 22 °C
METHOD OF FERTILISATION	SPONTANEOUS		FERTILISATION OCCURS WITHIN SPAWNING TANK
	SPAWNING		
EGG INCUBATION TANK SIZE	500-1000 L		
TIME TO HATCH	60 H		AT 22 ±0.5°C
LARVAE TANK SIZE	2000-10,000 L		INTENSIVE GREENWATER CULTURE
LARVAL YOLK-SAC PRESENT	0-3 DAH		AT 22 ±0.5°C
LARVAL FIRST-FEEDING	2-4 DAH		AT 22 ±0.5°C
			AFFECTED BY SURFACE SCUM, LIGHT INTENSITY, TURBULENCE, TEMPERATURE AND SALINITY
LARVAL SWIMBLADDER INFLATION	3-7 DAH		TIME TO METAMORPHOSIS IS DEPENDENT ON FACTORS AFFECTING GROWTH E.G.
METAMORPHOSIS	~ 10 MM TL		TEMPERATURE AND FEED AVAILABILITY
CANNIBALISM	~12 MM TL		SIZE GRADING IS NEEDED TO REDUCE INCIDENCE
WATER QUALITY PARAMETER	TARGET	DAH	ADJUSTMENT
pH	7.6 - 8.2	0+	
DISSOLVED OXYGEN (MG/L)	>6.00	0+	USE COMPRESSED OXYGEN DIFFUSER TO MAINTAIN SATURATION LEVEL
TEMPERATURE (°C)	22	0+	
SALINITY (PPT)	25 - 35	0+	
WATER EXCHANGE (%/DAY)	100 - 200	0+	INCREASE EXCHANGE AS LARVAE DEVELOP
SURFACE SKIMMER (HRS/DAY)	24	2+	MONITOR SKIMMER TO ENSURE LARVAE AT WATER SURFACE ARE NOT AFFECTED
PHOTOPERIOD (L:D)	(12:12) (18:06)	(0+) (6+)	INCREASE POST SB INFLATION
LIGHT INTENSITY (LUX)	225-400	0+	
GREEN-WATER (CELLS/ML)	1.4 x 10 ⁶	0+	PRO-AQUA* CONCENTRATE 57x10 ⁹ PER ML
LARVAL FEEDING SCHEDULE	TARGET	DAH	ADJUSTMENT
ROTIFER (R/ML)	20.0 - 5.0	4+	INITIAL 20/ML UNTIL FEEDING AND THEN INCREASE FREQUENCY OF REDUCED CONCENTRATION (E.G. 4x5/ML/D).
ARTEMIA (A/ML)	0.2 - 2.0	12+	0.2/ML UNTIL WEANED, THEN INCREASE CONCENTRATION AND FREQUENCY
WEANING DIET SIZE (µM)	200 - 800	18+	COMMENCE WEANING AT 10 MM TL

*Algae concentrate used Rotifer Diet-3600 (*Nannochloropsis/Tetraselmis* blend) from Reed Mariculture Instant Algae, imported via Proaqua Australia. <http://www.proaqua.net.au>

4. HEALTH MONITORING AND DISEASE PREVENTION, DIAGNOSIS AND TREATMENT

4.1 Introduction

Australian bass and mullocky are relatively hardy fish with relatively few disease problems when raised in good quality water in combination with other good husbandry practices. Both species are euryhaline and are capable of tolerating a wide range of naturally occurring water conditions, including variations in salinity, turbidity and temperature (see Chapter 1, Table 2 and Chapter 2, Table 6).

By contrast Yellowtail kingfish, as is typical of marine species, are stenohaline and ill equipped to deal with large environmental variations including fluctuating salinity, high turbidity, or pH outside the narrow range 7.5 to 8.5. They are however surprisingly tolerant of large, short to intermediate term declines in dissolved oxygen.

While many fish health problems can be avoided by careful attention to water quality and other good husbandry practices, once a disease problem occurs the cause must be identified as soon as possible. Once the cause of the disease is known, specific treatments can be used to reduce further losses and the economic impact of the disease. Constant routine measurement of water quality variables is essential as is vigilance in continually maintaining variables within tolerable limits. The maintenance and frequent reference to water quality records will help managers identify underlying factors that pre-disposed fish to disease and to take early action that may prevent recurrence or at least reduce the severity of the disease.

The severity of any disease outbreak depends on the interaction of three groups of predisposing factors illustrated in Fig. 93.

1. The fish themselves, especially their genetically determined natural susceptibility/resistance to particular disease agents. Disease susceptibility may vary substantially with the species, age or life cycle stage of fish and their previous exposure to the disease, including vaccination.
2. The culture environment. Provision of a poor or suboptimal diet and/or exposure to poor or suboptimal physiochemical environmental conditions, overcrowding and handling stress all influence susceptibility to disease.
3. The type and virulence/toxicity of the disease organism/agent

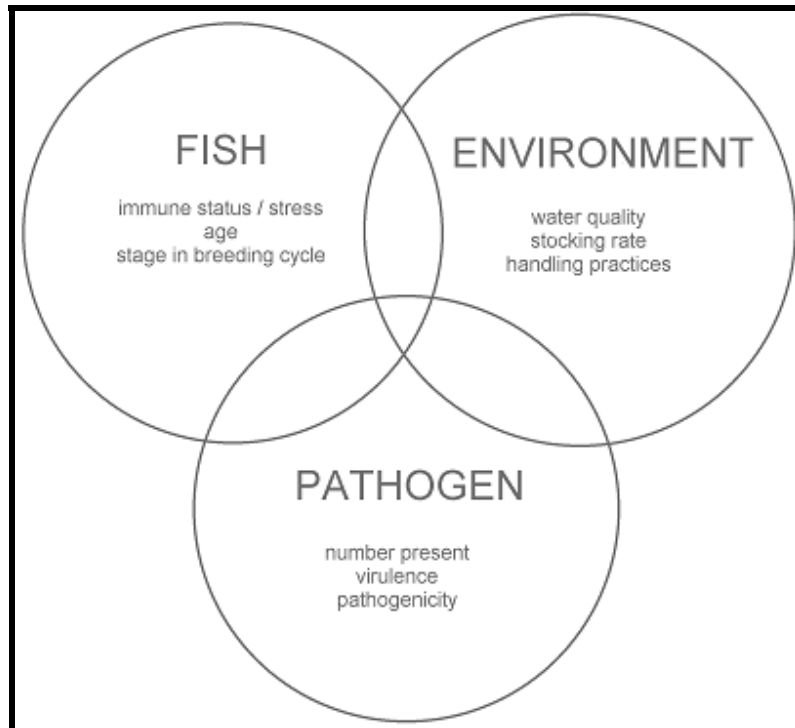


FIGURE 93: Diagram illustrating the interaction between the fish, the pathogen causing disease and the environment. (Source: Partridge et al., 2003).

Apart from a few catastrophically virulent diseases such as nodavirus infections of young immune-incompetent larval and juvenile fish (see Section 4.5 below), it is the interaction between these three factors that determine the severity of a disease or whether it occurs at all. For example, in the summer months, high water temperatures increase growth and food intake leading to increase waste production and thence reduced oxygen levels that compromise the general health and disease resistance of fish. Such increases in disease susceptibility are often coupled with increases in the replication rates of potential pathogenic microorganisms (bacteria, fungi and protozoans) thereby further exacerbating the likelihood of disease outbreaks (epizootics).

Important components of a successful health management strategy within fish hatcheries including those producing Australian bass, mullet and yellowtail kingfish are as follows:

- 1) Take all reasonable measures to exclude disease agents but be prepared with a health management plan if disease occurs to limit the impact and spread of disease within and beyond the hatchery.
- 2) If hatchery breeding stock need to be brought in, they must be *health certified* which means they must have a documented disease-free history and/or have tested negative when checked for disease carrier status in respect to specific pathogens where suitable tests are available.
- 3) If disease-free status cannot be established prior to receipt, incoming broodstock must be quarantined in a secure isolated area for 4 to 12 weeks of observation and subjected to prophylactic disease disinfection. (Quarantine is also recommended for health-certified broodstock brought in to the hatchery but a reduced period is satisfactory).

- 4) Risks of introducing disease with outsourced stock can be further reduced by separating different batches of fish. This precaution can be augmented by clever hatchery design, layout and operating protocols that minimise the probability of disease transmission via water and aerosols. Likewise, ensure that other potential routes (portals) of transmission such as on the skin and clothes of staff, on the surfaces of fish handling and treatment equipment such as buckets or water quality monitoring devices, are minimised using appropriate hygiene protocols. The latter must include mandatory routine cleaning, rinsing and disinfection of all facilities and equipment between successive uses and/or providing separate fully replicated (batch-dedicated) facilities, equipment, utensils and staff.

Disease prevention - Keep fish in optimum health and under minimum stress.

Healthy, well-fed fish kept in good quality water are much less likely to suffer from disease outbreaks. Many disease-causing organisms are commonly present in low numbers on the fish or in their environment. Healthy fish are more likely to withstand increases in the number of these potential pathogens in their environment. For broodstock held in controlled environment recirculation systems, disease prevention also entails disinfection of all new (incoming) seawater. The most appropriate of alternative seawater disinfection protocols, including ultra-filtration, chemical chlorination, UV treatment, ozonation or pasteurisation, depends on the disease agents being excluded, the susceptibility of host fish to such agents and the quality of source water and its accessibility in time, space and volume. As with seawater, pre treatment, including disinfection, of freshwater will vary in accordance with its source and quality. For example:

- Reticulated mains water should be routinely monitored for residual chlorine and de-chlorinated.
- Water sourced from streams or dams should be settled and /or pre-filtered to 1µm nominal and sterilised while degassing.
- Ground water must be regularly checked for, pH, excessive alkalinity, DO and other potentially toxic dissolved gases, especially carbon dioxide and hydrogen sulphide.

Frequently observe and become familiar with the normal appearance and behaviour of the fish.

Early recognition of disease often results in decreased losses as treatment and management practices can be fine tuned at an early stage in the disease process. Recognition of normal behavioural patterns and healthy appearance of fish is very important for early recognition and prevention or mitigation of impending health problems.

In juvenile and adult fish, altered feeding behaviour, particularly reluctance to feed vigorously, is often the first sign of trouble. Other behavioural signs of stress and onset of ecto-parasitic diseases include signs such as 'flashing' or a rapid rubbing movement on a surface of the tank; and in the case of gill parasites, gaping opercula, increased ventilation rates, "loitering" of the fish near the water inlets, 'hanging' over an air source. Other warning signs are changes in physical appearance such as dark coloured skin lesions and a 'hollow' or distended abdomen.

4.2 Larval Fish

As soon as the larvae start feeding, the following monitoring should be performed to assess health status. Quantitative evaluation of:

- predatory activity and feeding performance
- stress
- swim bladder development

4.2.1 *Predatory activity and feeding performance*

Presence and quantity of ingested food is the most important single indicator of fish health especially over the first 10 days of feeding. To do this a sample of 30-50 larvae should be sampled and microscopically examined according to the following schedule each morning an hour or so after the introduction of fresh live food.

- Pipette the larvae on a cavity or Sedgewick rafter slide
- Remove excess water with the pipette and put under the microscope
- Take a first look for body deformities not caused by handling.
- Observe the gut contents at x100 magnifications
- Look for whole rotifers or for their components such as mastax, lorica, or eggs.
- Look for other ingested material if any, and for internal/external parasites
- Look for the presence of calculi in the urethra and in the urinary bladder
- Record all findings on a specific form

Try not to damage the larvae with the pipette. Morphological condition assessment should be made as quickly as possible since heat irradiated by the microscope lamp causes shrinkage within minutes. The presence of ingested rotifers can be easily recognized by the presence of their masticatory mouth-parts (mastax), left undigested in the larval gut. Under a 100x magnification they clearly appear amongst rotifer egg and other debris. In practice only the number of mastax found is recorded to estimate the total number of rotifers eaten. The number of prey per larva ranges from 2-3 (early feeding) to over 50.

To check the ingestion rate of *Artemia* nauplii in older larvae, it is sufficient to visually estimate the percentage of repleted (fully fed) larvae sampled in a 100 ml transparent beaker by checking digestive tracts of the larvae for the presence of the deep orange nauplii that are visible through the transparent larval skin. Observation on prey ingested should be integrated with an assessment of fish behaviour as described below.

4.2.2 *Quantitative evaluation of stress*

Stress in fish larvae induces both morphological and behavioural changes that can be detected by the hatchery operator in order to improve culture conditions or to replace as soon as possible a poor larval batch. The main criteria for stressed fish larvae as described by Moretti et al. (1999) are:

- starvation
- calculosis (presence of deposits of calcium [usually as Ca oxalate] within the urinary duct)
- abnormally passive behaviour
- absence of "schooling" ("in the first two weeks of life")
- frenzied crowding at the tank surface perimeter (meniscus)

Starvation is an obvious indication that something has gone wrong. It is a general response to stress and often is impossible to link with a single cause. With the sole exception of acute toxicity to a chemical, all rearing parameters alone or more probably in association may stop larval feeding. As a starved fish will not survive for long, it is also important to monitor the onset of first feeding (see above). There is some scientific evidence that a direct correlation exists between environment-induced stress and the appearance of calculi in the urinary system of gilthead seabream and seabass larvae.

Although there is no confirmed correlation between calculi and death, calculi are often associated with starvation and consequently are present at a higher rate in dead larvae. The early appearance of calculi in a larval population is therefore considered as a stress indicator.

Calculosis can be easily detected by examining the lower part of the larval urinary duct (urethra and urinary bladder) under a microscope at 100 x magnification. This condition becomes evident by the appearance of a single stone-like corpuscle or a chain of smaller ones, reddish or grey in colour. Sometimes they completely obstruct the urethra. The count of renal calculi may be done when the repletion rate is being evaluated and can be recorded on the same sheet. When a large calculosis, say in more than 40% of the fish examined, is observed it can be interpreted as a sign of poor rearing conditions which typically will result in a low survival rate. In this case environmental and feeding parameters such as the following should be checked:

- excessive water currents due to wrong aeration or water inflow;
- disproportionate prey size;
- insufficient light intensity;
- dangerous levels of some water quality parameters such as dissolved oxygen and/or
- total ammonia nitrogen (TAN) content

Another useful indicator of stress is a high proportion of fish that do not actively swim and attack prey. At the mercy of water currents, the passive behaviour of stressed fish prevents efficient hunting of live food. Such abnormal behaviour can be easily detected as glimmering points of light throughout the rearing tank. This effect is caused by the retinas of the inverted passively drifting and tumbling fish as they catch and reflect artificial light sources. Such events may lead to the loss of a substantial part of a brood. An unhealthy rearing environment, together with a possible congenital factor is considered as the most probable causes of this syndrome.

By contrast, healthy broods of fish display the following normal predatory behaviour:

- Searching behaviour - continuous swimming with side to side head movement in search of food;
- Pointing and close approach to prey;
- strike preparation marked by tail flexion;
- attack marked by rapid tail straightening and forward thrusting to capture prey;
- Prey gulping;
- Resumption of searching

The exact location and density of larval shoals depends on:

- Water currents induced by aeration and water inflow;
- tank shape and size and water depth;
- active counter-current swimming of the larvae as a reaction to an external stimulus such as avoidance of high light intensity or high concentrations of live food.

Tanks of healthy fish comprise one or more dense shoals of larvae, typically in the calmest places of the tank or slowly moving about. Samples to monitor the size and health status of the population are taken inside these shoals. A particular type of behaviour such as an erratic swimming at the water meniscus should also be considered as a possible response to stress. This syndrome is characterized by a frenzied activity of larvae which seem to be attracted by the water meniscus where they get stuck, beating the tank wall head-on or being shaken by spasmodic head-up movements. This impairs their feeding rate and consequently survival. Usually only a small or insignificant fraction of larvae show these symptoms but over a protracted period, suggesting chronic stress or unsuitable rearing conditions as the cause.

Mass mortality can however occur with significant proportions of larvae exhibiting the “meniscus-stress syndrome” leading to death most commonly in the age range of between 10 and 30 days. If measures cannot be taken to counteract the stress inducing factors hatchery managers should consider the option of euthanizing the compromised population sooner than later and starting a new batch.

4.2.3 *Monitoring and control of swim bladder development*

At 18 -22°C, swim bladder formation begins within 3 to 4 days after hatch. The first sign of inflation clearly visible at 20-40x magnification after 5 to 7 days, is a single small air bubble within a tissue vesicle. A few days later, a second bubble develops and joins the first to form an almost spherical body that will gradually expand into an elongated vesicle.

Initial activation of the swim bladder relies on air gulping and swallowing ingestion at the tank surface. A temporary duct between the swim bladder area and the mouth (typical of physoclistic fish such as Australian bass, mullet and Yellowtail kingfish) makes this process possible. This active air swallowing is crucial to swim bladder development and if impaired, prevents proper swim bladder formation, as the duct remains open only for a few days. As experienced during the first 10 years of hatchery production of Australian bass (see Chapter 1) no or incomplete inflation of the swim bladder has severe consequences for developing larvae. These include spinal deformities, limited or negative buoyancy and abnormal swimming behaviour and hence greatly impaired feeding and growth. Even if the deformed fish reaches marketable size, its marketable value will be greatly diminished. Therefore an early correct determination of the percentage of

swim bladder inflation is vital to proceeding with a hatchery brood. These factors are important for normal swim bladder activation:

- physical barriers (scum) at the air- water interface;
- physio-morphological abnormalities in newly hatched larvae;
- early disease outbreaks;
- insufficient and irregular feeding;
- poor suitable water quality

Any scum or fine particulates at the air–water interface that prevents larvae from gulping air will impair swim bladder inflation. The presence of an oily layer originating from rotifers being fed an enrichment diet is a particular problem. Use of "surfaces skimmers" (see Chapter 1) largely mitigates this risk provided they are cleaned and checked that they are operating correctly regularly at least three times daily. Daily quantitative monitoring and recording of swim bladder development in 30-40 larvae should be done in conjunction with that of feeding status described above.

Monitoring should be continued until completion of inflation which varies according to fish species (from days 4 to 11 in AB, from days 3 to 7/8 in mulloway and from days 3 to 6/7 in YTK).

4.3 Diagnosis of Infective Disease – systematically investigate the cause of worrying changes in appearance, behaviour or increased mortalities.

The first step in identifying an infective disease agent in the hatchery is to observe and record disease symptoms and signs especially aberrant behaviour and appearance such as external colour or textural changes and presence and appearance of lesions. If practicable these recorded macroscopic observations should be supported with digital photographs and video recordings of affected fish in situ in rearing tanks of smaller vessels after capture and removal. Removed fish must also be subjected to a skin smear and gill biopsy. After being anaesthetised and or euthanized. The samples are smeared onto a glass microscope slide with a drop of water and examined under a microscope. For a comprehensive description of these biopsy procedures and identification of the likely pathogenic causes and organisms refer to the following manuals:

1. Herford A., and Rawling G. (1999) *Australian Aquatic Animal Diseases Identification Field Guide* - Publ. Department of Agriculture, Fisheries and Forestry, Canberra, Australia
2. Munday, B. (1996) *Treatment of finfish diseases. Fish Health Workshop* Post Graduate Foundation in Veterinary Science, the University of Sydney.
3. Noga, E. J. (1996) *Fish Disease: diagnosis and treatment*, Mosby, St Louis.
4. Read P., Landos M., Rowland S.J., & Mifsud C. (2007) *Diagnosis, treatment and prevention of the diseases of the Australian freshwater fish Silver Perch (Bidyanus bidyanus)* Publ. NSW Department of Primary Industries ISBN 978 0 7347 1792
5. Thorne T. J. (1995) *"Fish Health for Fish Farmers in Western Australia"* Fisheries Department of Western Australia.
6. Untergasser, D. (1989) *Handbook of Fish Diseases*, TFH Publications Inc.

Whether or not on-site examination reveals a possible causative agent, the relevant state department Aquatic Health Unit should be contacted and live or moribund fish, plus suitable preserved samples, delivered to them as soon as possible for a definitive diagnosis and recommended courses of treatment, eradication and future prevention.

4.4 Disease Treatment (refer to item of references listed above and Table 11 below)

4.4.1 General

Once the cause of an infective disease has been positively identified, an appropriate course of treatment (see Table 11) can be initiated.

Many different treatments are often suitable for the treatment of a specific disease problem. The final choice of treatment will depend on many factors including the pattern of mortality and condition of remaining fish, water temperature, stocking density, type of filtration system, value of the fish and the cost and availability of the treatment chemical. Many fish with early stages of disease do not eat, making "in feed" treatments less effective. In addition, many fish with early stages of severe diseases will die irrespective of which treatment is used. The priority is to prevent more fish from becoming infected and to treat those with mild disease. Until confidence and experience has been gained in identifying and treating disease problems commonly occurring in a hatchery, it is wise to consult a more experienced person or organisation before starting treatment. It should be noted that many chemotherapeutics require prescription from a registered veterinary surgeon.

Some treatments used successfully at finfish hatcheries for various disease-causing agents are summarised in Table 11. As some treatments are stressful to fish, especially diseased fish held in warmer water, it is advisable to test the treatment on a small group of fish before treating the larger group. Good aeration, preferably in the form of pure oxygen should always be administered during treatments. Tanks should be vacuumed to remove various infective stages of pathogens after each treatment.

All chemicals should be used with great care when administering to diseased fish. Read instructions pertaining to the use of these chemicals prior to application and ensure that appropriate protective clothing and equipment is available and used.

In Australia, the Australian Pesticides and Veterinary Medicines Authority (APVMA) is responsible for regulating chemicals used in agriculture, including aquaculture. Other relevant legislation in NSW includes the *Stock Medicine Act 1989* and the *Food Act 2003*. Hatchery operators should be aware of the rules and regulations of these Acts and any amendments in order to use chemicals legally, responsibly and safely. Information regarding the use of chemicals in aquaculture can be obtained at www.apvma.gov.au (Rowland et al., 2007).

A small number of chemicals are registered for use in aquaculture in Australia. To be approved for use in food animals, a drug must usually undergo rigorous testing of its efficacy in treating specific diseases in each species at specific dosages and routes of administration. Information must be obtained on residue dynamics, safety for the operator and consumer, and any environmental effects. This can be time consuming and expensive. Once completed, the registered drugs must be used only in accordance with the label to treat the species on the label, at the directed dose rates.

The APVMA, can allow the use of unregistered chemicals, or registered chemicals off-label, under a minor use permit (MUP). When no alternative registered chemical is available, an MUP can be issued temporarily following the APVMA completing a risk assessment. It is essential to confirm with the APVMA the applicability and validity of any MUP (via the APVMA website) and/or I&I NSW (or equivalent department in other states) the conditions under which a chemical can be used.

Salinity change or a formalin bath has controlled the majority of the fish health problems that have been encountered with Australian bass and mullet. A freshwater bath for euryhaline fish is the treatment of choice for some disease agents such as metazoan and protozoan parasites as it is cheap and does not leave toxic residues in the tissue. For AB and mullet held in full strength seawater (35 ppt), a fresh water bath of less than 2 ppt for 90 minutes is sufficient to kill or remove the majority of external parasites on the fish. Freshwater baths are not however effective in treating fungal or bacterial diseases and can't be used at all for high salinity marine (stenohaline) species such as YTK.

For Australian bass and mullet held in low salinity, a bath in high salinity water will be more effective than fresh water. A detailed description of pathogens and treatments is available in many texts as listed above and these should be consulted prior to treatment. Understanding the lifecycle of the disease agent and factors that can be exploited to reducing infection levels help when deciding the type and frequency of treatment options that may be used.

TABLE 11: Infectious diseases of hatchery held, AB, mullet and YTK and recommended control and prevention measures (Source: OIE Manual of Diagnostic Tests for Aquatic Animals, 2006).

DISEASE	CAUSATIVE AGENT	TYPE OF DISEASE AGENT	SYNDROME & SYMPTOMS	CONTROL & PREVENTION MEASURES
Viral nervous necrosis (VNN)	Encephalitis virus (LeEV) – a beta-noda-virus	Virus	Encountered in hatchery production of Australian bass, mullet larvae and advanced YTK. Pale or dark colouration; erratic swimming behaviour; spiral swimming; boating; ‘fainting’, extensive vacuolation of the brain & spinal cord; generally encountered during hatchery phase. Asymptomatic carriers also common.	No treatment developed to date. Prevention by screening of broodstock; low larval rearing densities; optimal larval nutrition; improved broodstock nutrition; improved hatchery hygiene including sterilisation of incident seawater and full dry-out and disinfection of plant and equipment between successive hatchery broods especially once a disease outbreak has been experienced.
Lymphocystis	Lymphocystis virus	Virus	Wart-like growths on skin & fins; generally only fatal if infection severe & associated with very poor environmental conditions.	Removal of infected fish; improved environment.
Vibriosis	<i>Vibrio harveyi</i> ; <i>Vibrio</i> spp.	Bacteria	Marine fish with darkening; lethargy; anorexia; reddened ulcerations on body; reddened abdominal fluid; associated with nursery systems, poor environment & skin trauma.	Improved environment; antibiotic treatment as prescribed by a qualified veterinarian or fish pathologist e.g. Oxytetracycline (in feed at 75 mg/kg biomass or in water at 100-200 mg/L) otherwise trimethoprim or oxolinic acid.
Bacterial haemorrhagic septicaemia	<i>Aeromonas hydrophila</i> ; <i>A. sobria</i> ; <i>A. Caviae</i> ; <i>Aeromonas</i> spp.; <i>Pseudomonas</i> spp.	Bacteria	Freshwater fish with irregular reddened skin ulcerations; lethargy; anorexia; reddened abdominal fluid; pale gills; associated with poor environment & skin trauma.	Improved environment; antibiotic treatment as prescribed by a qualified veterinarian or fish pathologist e.g. Oxytetracycline (in feed at 75 mg/kg biomass or in water at 100-200 mg/L) otherwise trimethoprim or oxolinic acid.
Integumentary bacteriosis	<i>Aeromonas sobria</i> ; <i>Aeromonas hydrophila</i> ; <i>Vibrio harveyi</i> ; <i>Vibrio alginolyticus</i>	Bacteria	Irregular reddened skin ulcerations; loss of scales; associated with poor environment & skin trauma.	Improved environment; increased water exchange.
Streptococcosis	<i>Streptococcus iniae</i>	Bacteria	Darkened fish; anorexia; pale gills; reddened abdominal fluid; reddened abdominal organs & inner wall.	Antibiotic treatment. Vaccines are not available for Australian bass, mullet or YTK, but are being developed for Striped bass.
Columnaris disease	<i>Flavobacterium columnare</i> ; <i>Flavobacterium johnsoniae</i> ; & <i>Flavobacterium</i> sp. (gliding forms) in freshwater <i>Tenacibaculum maritimum</i> in seawater	Bacteria	Pale skin patches on dorsal surface behind dorsal fin & on caudal peduncle; lethargy; most commonly occurs in nursery phase; in older juveniles a mouth form with erosion of skin around upper & lower jaws has been seen; associated with overstocking, tank rearing, poor hygiene & skin trauma.	Treatment in potassium permanganate or copper baths 0.28 mg/ L Cu++ 24h or 0.1 to 0.2 mg/L Cu++ 10 days may help in early disease NB Use chelated copper rather than CuSo4 on fish in seawater; Otherwise antibiotic treatment as prescribed by a qualified veterinarian or fish pathologist
Bacterial gill disease	Various bacteria, <i>Flavobacterium</i> spp., <i>Cytophaga</i> spp.	Bacteria	Swimming at water surface; gulping; rapid opercular movement; excess mucus on gills; white patches on gills; most commonly occurs in nursery phase.	Improve water quality; treatment with salinity reversal, or under veterinary advice use of potassium permanganate or quaternary ammonium baths; increase water exchange; reduce stocking density.
Bacterial peritonitis	Various Gram-negative & Gram-positive bacteria including <i>Vibrio harveyi</i> & <i>Aeromonas hydrophila</i>	Bacteria	Darkened fish; lethargy; swollen abdomen; adhesions & bad smelling fluid in abdomen; abdominal fistulas; more common in recirculation systems.	Cull affected fish; antibiotic treatment prescribed by a qualified veterinarian or fish pathologist e.g. Oxytetracycline (in feed at 75 mg/kg biomass or in water at 100 -200 mg/L) otherwise trimethoprim, or oxolinic acid.

DISEASE	CAUSATIVE AGENT	TYPE OF DISEASE AGENT	SYNDROME & SYMPTOMS	CONTROL & PREVENTION MEASURES
Bacterial enteritis	Various Gram-negative bacteria	Bacteria	Acute disease in intensive larval rearing systems; anorexia; pin heads; darkened fish & death.	Cull affected larval batch
Fin and tail rot	<i>Aeromonas</i> spp.; <i>Pseudomonas</i> spp.; <i>Vibrio</i> spp.; <i>Flavobacterium</i> spp.; <i>Cytophaga</i> spp.	Bacteria	Erosion of soft tissue in fins and tail; may extend to involve entire tail & caudal peduncle.	Improve environment; reduce stocking density
Epitheliocystis	Epitheliocystis organism – a <i>Chlamydia</i>	Bacteria	Swimming at water surface; rapid opercular movements; disease rare but seen in marine fish & in recirculation systems.	None known
White spot	<i>Ichthyophthirius multifiliis</i> in freshwater <i>Cryptocaryon irritans</i> in marine	Protozoa	'Flashing'; rubbing skin on surfaces; anorexia; swimming at water surface; white spots on skin & fins.	Treatment with salinity reversal, formalin baths at 100-200 ppm for 1 h, or Formalin at 75g/l for 2h or 25g/l for combinations; treatment in copper bath for marine fish 0.28 mg/ L Cu++ 24h or 0.1 to 0.2 mg/L Cu++ 10 days NB Use chelated copper rather than CuSO ₄ on fish in seawater
Chilodonelliasis	<i>Chilodonella</i> spp.; <i>Chilodonella hexasticha</i>	Protozoa	Swimming at water surface; rapid opercula movement; flared opercula; seen in poor environmental conditions & in weakened fish.	Treatment with salt, formalin at 100-200 ppm for 1 h, or Formalin at 75g/l for 2h or 25g/l for 24h or potassium permanganate bath or combinations
Trichodiniasis	<i>Trichodina</i> complex spp.	Protozoa	Swimming at water surface; rapid opercular movements; excess gill mucus; typically follows cold water temperatures, high organic loads & high stocking densities.	Increase water exchange; treatment with salt or formalin bath at 100-200 ppm for 1 h, or Formalin at 75g/l for 2h or 25g/l for 24 h.
Ichthyobodosis (costiasis)	<i>Ichthyobodo necator</i>	Protozoa	'Flashing'; rubbing skin on surfaces; opaque patches on skin; raised scales; swimming at water surface; rapid opercular movements; flared opercula.	Treatment with salinity reversal; or formalin at 100-200 ppm for 1 h, or Formalin at 75g/l for 2h or 25g/l for 24h or potassium permanganate bath.
Piscinoodiniasis	<i>Piscinoodinium</i> sp.	Protozoa	Found on AB and Mulloway in freshwater: In young fish opaque patches or a greenish discolouration of the skin; patches of skin lifting of surface & ulcers in older fish; rapid opercular movements; excess gill mucus; dark green gill colour.	Treatment with salt bath for AB and mulloway currently held in fresh water.
Velvet Disease = Amyloodiniasis	<i>Amyloodinium ocellatum</i>	Protozoa	Found in marine conditions: In young fish opaque patches or a green discolouration of the skin; patches of skin lifting of surface & ulcers on older fish; rapid opercular movements; excess gill mucus; dark green gill colour More common in broodstock and in raceways; associated with low water temperatures or rapid drops in temperature.	Fresh water bath for AB and mulloway held in seawater. Otherwise hydrogen peroxide (75g/L) bath for 75minutes (see further treatment details below) .

DISEASE	CAUSATIVE AGENT	TYPE OF DISEASE AGENT	SYNDROME & SYMPTOMS	CONTROL & PREVENTION MEASURES
Gill fluke	<i>Diplectanum</i> sp.; <i>Dactylogyrus</i> sp.	Monogean trematodes	Rapid opercular movements; anorexia; white areas on gills.	Treatment with salinity reversal for AB and mullocky, or formalin at 100-200 ppm for 1 h, or Formalin at 75g/l for 2h or 25g/l for 24h or praziquantel bath 1-2 ppm for 24h.
Skin fluke	<i>Neobenedinia melleni</i> ; <i>Gyrodactylus</i> spp.	Monogean trematodes	Marine fish with opaque cornea; white patches on skin; skin ulcers; associated with high salinity & cool water temperatures.	Treatment in freshwater for AB and mullocky or praziquantel bath 1-2 ppm for 24h.
Myxosporidiosis	<i>Henneguya</i> sp.; <i>Kudoa</i> sp.	Spore-forming protozoa	Disease uncommon but histologically spore cysts seen in gill filaments (<i>Henneguya</i> sp.) & brain (<i>Kudoa</i> sp.).	None known
Microsporidiosis	<i>Pleistophora</i> sp.	Spore-forming protozoa	Raised lumps on skin; soft white nodules in muscle.	None known
Integumentary mycosis	<i>Saprolegnia</i> spp.; <i>Achlya</i> spp.	Fungi	Raised, fluffy growths on skin & fins; associated with low water temperatures & skin trauma.	Salinity reversal and formalin baths formalin at 100-200 ppm for 1 h, or Formalin at 75g/l for 2h or 25g/l for 24h ; do not handle fish when water temperatures low.
Branchiomycosis	<i>Brachyomyces</i> sp.; <i>Achlya</i> spp.	Fungi	Swimming at water surface; rapid opercular movements; white & red patches (mottled appearance) on gills; associated with cold water temperatures & high organic loads.	No treatment known; reduce organic load & increase water exchange.
Fish louse	<i>Argulus</i> sp.	Copepod	Disc-shaped parasite visible on skin; red foci; darkening.	Treatment in organophosphate especially Trichlorphon at 0.5 ppm bath Leave fish in dose water where active ingredient will fully denature after about 24 hours.
Anchor worm	<i>Lernaea</i> sp.	Copepod	Thin body of female parasite visible on skin with small red ulcer where parasite penetrates skin.	Treatment in organophosphate especially Trichlorphon at 0.5 ppm bath Leave fish in dose water where active ingredient will fully denature after about 24 hours.

4.4.2 Management of velvet disease and viral nervous necrosis

Two diseases that present continuous high level and widespread risk to hatchery production of finfish including AB, mullet and YTK are velvet disease and viral nervous necrosis (VNN).

- Velvet Disease - *Amyloodinium ocellatum* (based on Fielder et al., 2008)

By far the most common disease of AB, mullet and YTK and indeed of all farmed fresh, brackish-water and marine finfish globally, velvet disease, is caused by the single celled (protozoan) *Amyloodinium ocellatum*. In common with other parasitic “Dinoflagellates”, *A. ocellatum* is distinguished by a motile life stage possessing 2 flagella, one a regular backward beating form, the other ribbon-like that beats to the cell’s left.

As illustrated in Fig. 94, *A. ocellatum* has 3 life stages:

1. The trophont (2 in Fig. 94). This is a pear shaped (pyriform) parasitic feeding stage commonly averaging 100-150 µm with individuals occasionally up to 350 µm). Trophonts attach to and feed mainly on the epithelial (skin) cells of the gills but also of the scales and eyes of infected fish via root like structures (rhizoids) on the base. Duration of infection in the trophont stage increases from 1 to 5 days with temperature over the range 14-27°C. At this point the rhizoids are retracted and the trophonts drop from the host fish to the substrate on the floor of ponds and rearing vessels.
2. The tomont (3 to 6 in Fig. 94), This is an external stage that after changing from the previous pear shape to spherical, begins cell division on the substrate after about 12 hours and continues Fig. 95).
3. Dinospores (7 in Fig. 94), these are a free swimming infective stage released from sub-cells that have undergone sporulation

4.4.3 Modes of entry and symptoms of *A. ocellatum* disease outbreaks

In aquaculture systems, where stocking density is high fish are usually more susceptible.. Entry of the pathogen into a hatchery can lead rapidly to high intensity infections.. Entry pathways of the pathogen into the facility may be introduced stock, contaminated equipment, influent water or even aerosols.

Clinical signs of infection usually manifest as parasite intensity increases rapidly. Loss of appetite (anorexia) is a key indicator of chronic *A. ocellatum* infection at low intensity levels. Other signs of infection include flashing, irregular or rapid opercular beat and uncoordinated movement. Unfortunately, these clinical signs are often encountered in many other parasitic infections and are therefore not diagnostic for *A. ocellatum*. The gills are considered the primary attachment site for *A. ocellatum* infections. However, infections can also occur on skin and eyes. Attachment to the host epithelia with rhizoids can cause physical damage to several cells surrounding each trophont. In, heavy infestations this damage to the gills can result in anoxia and impaired osmoregulation and heavy mortality, within as little as 12 hours.

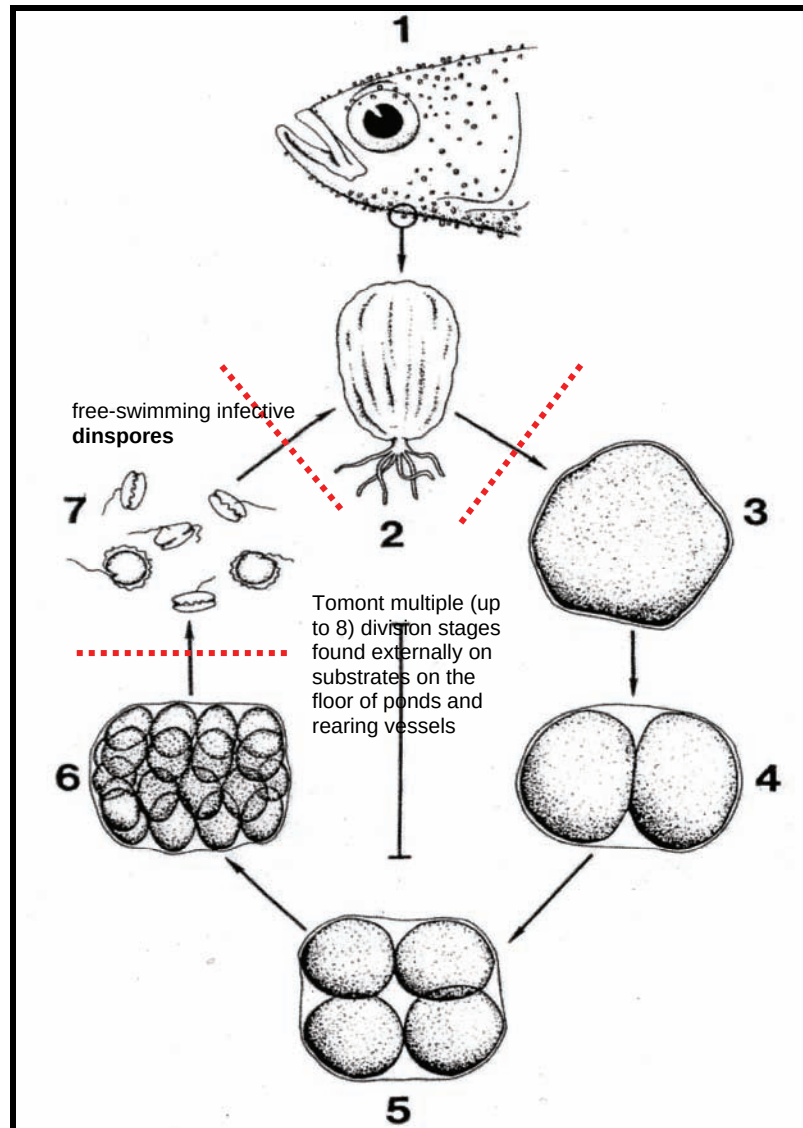


FIGURE 94: Life stages of *Amyloodinium ocellatum*. 2 attached trophont stage; 3-6 multiple division tomont stages ; 7 free swimming dinospores. (Source : Lom and Dykova, 1992).

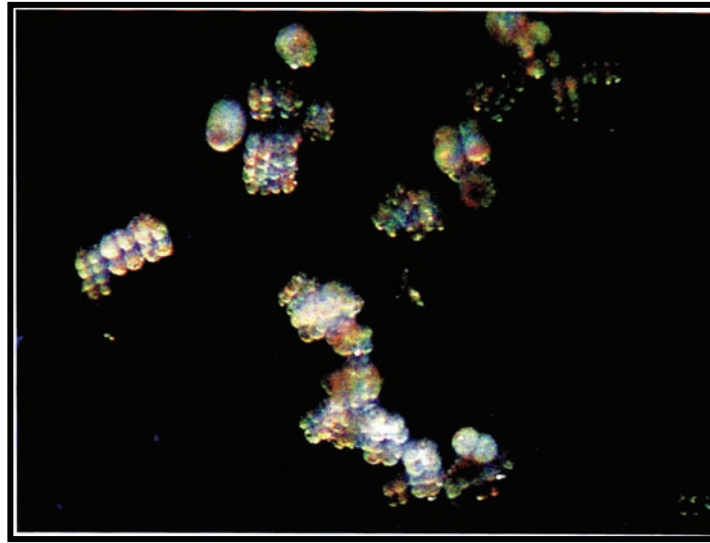


FIGURE 95: Photomicrograph of Tomont stages of *Amyloodinium ocellatum* in various stages of division. (Source: Roberts-Thomson, 2008).

4.4.4 Management (prevention, control and treatment) of Velvet disease

Management of *A. ocellatum* in aquaculture facilities is a difficult task. The parasite itself is extremely hardy being able to reproduce in salinities ranging from 3 ppt up to 45 ppt and temperatures from 17°C up to 40°C, making it more resistant to environmental change than many of its hosts including AB, mullet and especially YTK. Under optimum temperatures of 17-23 °C, the life cycle can be completed in as little as 5 days. Treatment also becomes problematic when targeting the tomont stage. This is insulated from the environmental conditions by its cyst wall, making it near impervious to chemicals. Despite these hurdles, a measure of success has been reached in the implementation of chemotherapeutic treatments.

Chemicals affecting the trophont stage are generally non-specific for *A. ocellatum*. These include formalin, copper compounds, hydrogen peroxide, and fresh water baths. While these treatments are effective in shocking the parasite from its host they do not generally arrest development and trophonts through to tomonts. Many of these compounds are also toxic to the fish, making them generally ineffective as control measures. Copper compounds can be useful in dinospore elimination. Chloroquine, a common human anti-malarial drug, has also been found effective however, its high cost and long half-life in fish flesh limit its cost effectiveness to hatcheries in the treatment of broodstock and intensively reared juveniles. While malachite green, acriflavine, furanace and nitrofurazone have been found to act on the tomont stage, none are acceptable treatments due to their adverse affects to human health, including hatchery staff.

Securing hatchery facilities against intrusion of *A. ocellatum* therefore remains the best method of diseases prevention and control. This entails:

1. quarantining and regular prophylactic treatment of broodstock
2. physical separation of larval and juvenile rearing systems and associated utensils and equipment and replicate sets thereof
3. routine cleaning and chlorine disinfection of rearing tanks and equipment between successive uses
4. disinfection of all incoming freshwater and seawater

A large array of alternative treatments (Fig. 96 and Table 12) are effective in the short-term management of *A. ocellatum* infections. Freshwater bathing is particularly cheap and effective for treating euryhaline fish species such as the bass and mullet. However, freshwater does not eliminate all trophonts from the gills and therefore remaining trophonts can potentially re-infect if the original salinity is restored, even if fish are moved to a different tank or pond.

Hydrogen peroxide added to rearing water at 75 g/L for 30 minutes is the treatment of choice in hatcheries as it is relatively cheap, leaves no flesh residues and is very effective for treating both euryhaline fish, such as AB and mullet, and marine fish such as YTK. It is especially effective due to its ability to shock trophonts from fish gills and skin and its suppression of dinospore production and release from tomites when used over a protracted period of 4 days. Repeat dosing may however be needed in such protracted treatment due to the rapid break down of hydrogen peroxide in the environment. Caution needs to be exercised when using hydrogen peroxide due to its potential negative impacts of treated fish including reduced feeding and growth for up to three weeks.

The overall efficacy of agents that simply remove trophonts from infected animals such as freshwater, formalin and short duration hydrogen peroxide bath, will ultimately be determined by bio-security procedures in place within a facility. If treatment is not coupled with movement of treated fish into new tanks, or tank brushing and vacuuming to remove the released tomites from the system, then the lifecycle of the parasite will not have been effectively disrupted and re-infection is almost certain.

TABLE 12: Tomont chemotherapeutic results including tomont division, dinospore emergence and motility from vitro trials.

Treatment	Dosage (mg/l)	Treatment time (mins)	Tomont Division	Maximum divisions	Dinospore Emergence	Dinospore Motility
Bronopol	50	30	Y	6	Y	Y
Bronopol	50	4 days	Y	6	N	N/A
Hydrogen Peroxide	200	30	Y	7	Y	N
Hydrogen Peroxide	200	4 days	Y	2	N	N/A
Hydrogen Peroxide	75	30	Y	7	Y	N
Hydrogen Peroxide	75	4 days	Y	2	N	N/A
Formalin	200	60	Y	6	Y	Y
Formalin	25	4 days	Y	5	Y	Y
Marine Oodinium and Whitespot Treatment	150	4 days	Y	4	N	N/A
Control	-	4 days	Y	8	Y	Y

Recommended treatment highlighted. (Source: A. Roberts-Thompson, 2008).

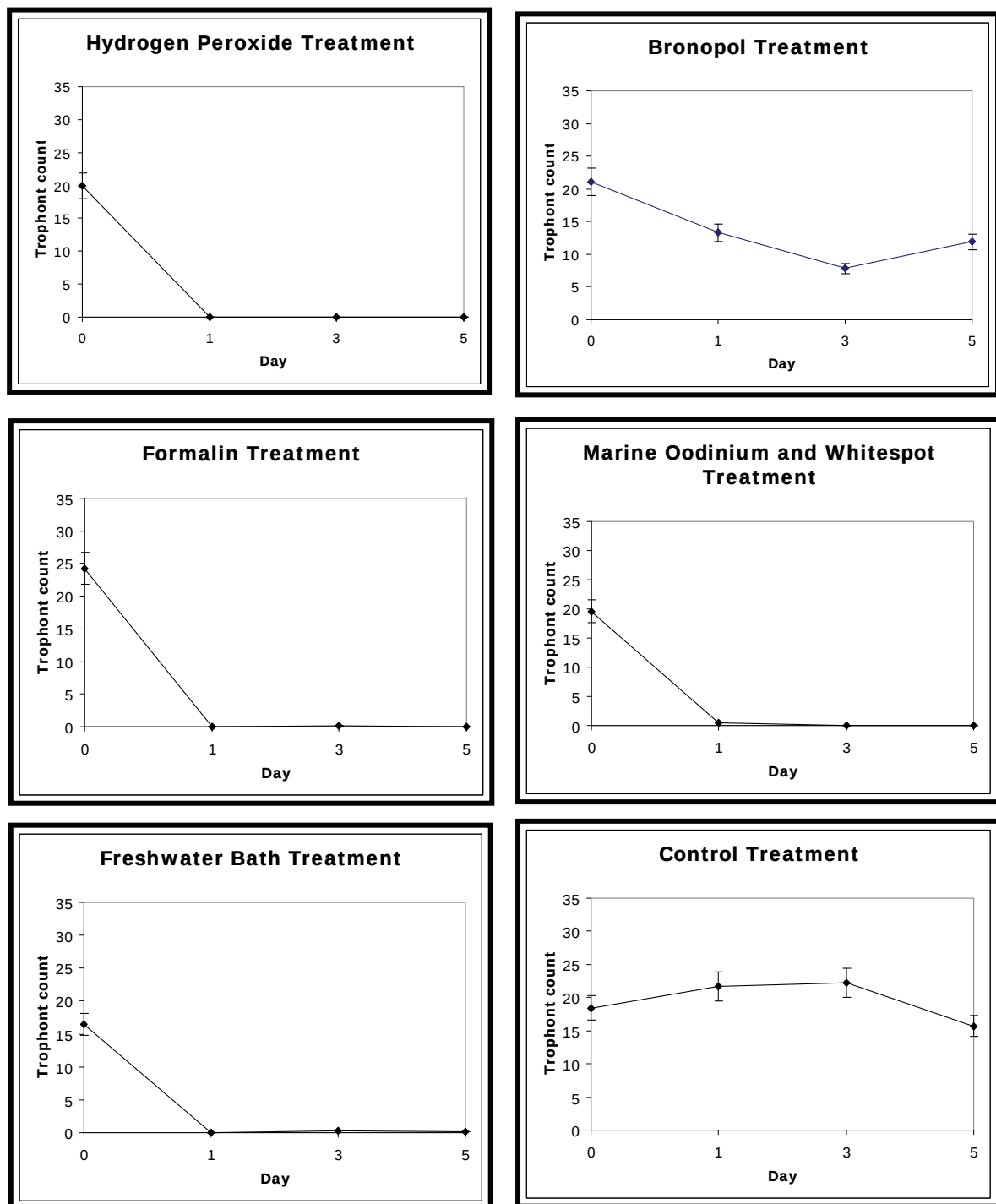


FIGURE 96: Trophont treatment trials with a range of chemotherapeutic chemicals of barramundi infected with *A. ocellatum*. (Source: A. Roberts -Thompson, 2008 in Fielder et al., 2008).

4.5 Viral Nervous Necrosis (VNN = Viral encephalopathy and retinopathy)

4.5.1 Introduction

Viral nervous necrosis (VNN) or viral encephalopathy and retinopathy (VER) caused by a betanodavirus was formerly called barramundi picorna-like virus when first encountered in Australia in 1989 (Glazebrook, Heasman & de Beer, 1990). VNN is a serious disease of finfish that has been reported in over 40 fish species from most tropical to temperate parts of the world, with the exception of Africa. Most reports of disease have been from farmed fish, with disease observed most commonly in larval and juvenile fish although disease in adult fish has been reported. Mortality rates of up to 100% are observed in larval fish populations with rates tending to decrease as the size/age of infected fish is increased. Fish surviving infection can become sub-clinical carriers and vertical transmission is suspected (although little evidence is available to support the suspicion) to occur via spawning products from sub-clinically infected broodstock to progeny during spawning.

The aetiological agent causing VNN is a virus of the genus *Betanodavirus* (is the target for detection of viral RNA by PCR). The viral coat protein is the target for detection by immunological methods. There are four recognised genotypes all the other known Australian isolates are members of the RGNNV genotype. However, the isolates form two distinct groups within this genotype; one group comprising isolates from New South Wales and South Australia and a second group comprising isolates from Queensland, the Northern Territory and Tasmania.

Clinical signs of disease include:

- colour change - affected AB become lighter
- abnormal swimming behaviour, including spiral and/or looping swim pattern, uncoordinated darting, belly-up at rest and pinpoint flashes of light reflected by the retinas of afflicted fish as they are roll ,twist and turn
- overinflated swim bladder
- lethargy and anorexia leading to emaciation
- blindness resulting from vacuolation lesions in eyes
- abrasions

These may vary depending on the host species but result from damage to the brain and retina, i.e. anorexia (a secondary effect of blindness), abnormal swimming and changes in body colour.

Gross pathological lesions are uncommon, but over-inflation of the swim bladder in infected sevenband grouper and red drum has been reported. In larval epizootics, mortalities of up to 100% cause problems for hatchery productivity and the regular supply of fingerlings for on-growing. While high level mortalities in intensive larval cultures are most common, there are also records of disease in older juveniles. Such reports indicate that nodaviruses can cause significant mortalities in any class of cultured marine finfish.

In the absence of chemotherapy for viral diseases of fishes there is the potential for significant economic loss. More recently around the world reports of VNN in fish reared in freshwater have occurred: European sea bass, sturgeon, European eels and Chinese catfish and freshwater guppy. These reports confirm the possibility that natural outbreaks of VNN and nodavirus infections can occur in farmed freshwater fishes. Also there is the suggestion that spread of nodavirus can occur between different species of fish in the fresh water ecosystems. The lack of epidemiological information on the prevalence of nodavirus in wild populations remains a constraint on the development of appropriate policy for managing real risks in fisheries.

In Australia, disease was first reported from barramundi (*Lates calcarifer*) larvae in the late 1980s (Glazebrook, Heasman & de Beer, 1990). In the following years, high mortalities were seen in the first few weeks of experimental, intensive larval culture. VNN epizootics had a significant effect on the one commercial barramundi hatchery operating at that time, but were rare in government hatcheries. Changes in husbandry and improved hygiene practices in the hatchery resulted in prevention of any further VNN epizootics. Subsequently, in 1999, one commercial barramundi hatchery experienced mass mortality of barramundi around 15 days of age in larval rearing ponds. This was the first time any significant VNN was seen outside of intensive larval rearing systems. Since then nodavirus has been reported from an increasing number of species including Australian bass (*Macquaria novemaculata*), mullet (*Argyrosomus japonicus*), yellowtail kingfish (*Seriola lalandi*), barramundi cod (*Cromileptes altilevis*), goldspotted rockcod (*Epinephelus coioides*), flowery cod (*Epinephelus fuscoguttatus*), sleepy cod (*Oxyeleotris lineolatus*) and striped trumpeter (*Latris lineata*) from marine and freshwater facilities in New South Wales, Northern Territory, Queensland, South Australia and Tasmania. As other species are evaluated for aquaculture potential, the range of species found to be susceptible to infection is likely to increase.

Nodaviruses have been detected in juvenile fish surviving experimental and natural infections and while the duration of viral persistence is unknown, virus has been reported in one fish species that survived acute infection for at least 12 months after the initial disease outbreak. Virus has also been detected in healthy juvenile and adult fish from susceptible species and from species in which disease has not been observed. Antibodies have been detected in broodstock yet transmission via spawning products from infected broodstock to progeny is still considered the most common mode of transmission. Thus, the relationship between immune status and infectivity remains to be determined.

Because the effect of Australian nodavirus on native fish species is unknown, strict controls are in place to reduce the risk of translocation of virus with commercial stock or stock used for restocking programs and to reduce the risk of escape of virus from aquaculture facilities into the environment. Exclusion of the virus from aquaculture premises, good hygiene in these premises and reduced stocking densities have decreased the incidence of VNN outbreaks. It is worth noting that while reduced stocking densities can undoubtedly help they are not necessarily an essential component of a risk reduction strategy (Schipf, pers. comm.). It should be noted that currently no reliable non-destructive test is available for screening broodstock fish for nodavirus status. Blood and even gonad tissue have proven to be poor samples to determine nodavirus status of AB, as indicated by many negative results from blood from fish which were confirmed infected by nodavirus in brain and retina tissue sample.

Betanodavirus infection has a significant economic, social and environmental impact in Australia through direct losses due to disease, inhibition of trade for established and emerging aquaculture industries, restriction on locations suitable for aquaculture expansion, and suspension of fish restocking programs due to concerns of the impact on native fish species due to translocation of the virus with stock.

4.5.2 Management (Prevention control and eradication) of VNN:

Preventative Strategy comprises 5 components (Chapter 1. Viral Diseases FAO Gilda D. Lio-Po and Leobert D. de la Pena)

- Screening of all broodstock for VNN free status. (This is currently not possible for Australian bass, mullet and YTK. The development of reliable, non-destructive tests such as PCR and / or ELISA is essential to allow broodstock screening)
- strict hygiene coupled with isolation of broodfish from hatchery and nursery rearing areas.
- ozone disinfection of all incident seawater used to incubate eggs and to rear larvae and early juveniles
- ozone disinfection of embryos
- vaccination

Eradication Strategy - Total disinfection of hatchery - in response to an initial major catastrophic VNN disease outbreak or subsequent succession thereof.

Preventative Strategy

1. Screening for VNN free status and quarantining of all broodstock.(see above comments regarding inadequate test and the need for caution accepting results from blood samples).

Pre- and post-spawning screening of broodstocks for VNN using a PCR test is very important. Only VNN-negative broodstocks should be retained and allowed to spawn. This precaution is very effective in preventing vertical transmission of the virus when combined with disinfection of the fertilized eggs preferably using ozone.

2. Strict hygiene coupled with isolation of broodfish from hatchery and nursery rearing areas.

Strict hygiene is very important in the management of VNN infection. Betanodaviruses are quite resistant to some environmental parameters, thus it is highly possible that the virus could be easily translocated via contaminated rearing water and other hatchery paraphernalia. The use of non-recycled, chemically treated rearing water and decontamination of tanks after every hatching cycle were effective in preventing VNN infection in Australian barramundi hatcheries.

Contrary to earlier fears that live feeds, rotifers and brine shrimp were a potential source of infection they in fact appear to be insusceptible to betanodaviruses. Another potentially important issue in managing this disease is that some species of fish such as grouper have been shown to be more susceptible to VNN at higher water temperatures, i.e temperature may differentially affect disease and host in this case favouring the virulence of the disease (VNN) over the resistance of the host (finfish larvae and early juveniles)

3. Ozone disinfection of all incident seawater used to incubate eggs and to rear larvae and early juveniles.

The amount of ozone required to inactivate NNV and other potential viral diseases such as infectious pancreatic necrosis virus (IPNV) is only 0.1-0.2 mg/ liter of seawater for 1 - 2.5 minute. However the recommended protocol based on the recent work of Battaglene and Morehead, 2006) to disinfect Day 3 post-fertilisation embryos of striped trumpeter is 1 mg ozone/L seawater for 1 minute (CT = 1). Similarly, research by Ballagh et al. (2010) has shown that ozone at a CT of 1 is the maximum concentration that mullet eggs can tolerate without causing damage to the eggs which prevents successful hatching. This protocol not only safeguards against betanodavirus but reduces bacterial loads and improves incubator hygiene. Since adopting the practice of ozone disinfection of embryos, and other control measures, there have been no mortalities attributed to betanodavirus in cultured striped trumpeter.

4. Other measures in the control of VNN are:
 - a. Cleaning, chemical disinfection and dry out of all hatchery buildings plumbing and other equipment and utensils with caustic soda, gluteraldehyde or chlorine ;
 - b. rearing of each batch of larvae and juveniles in separate tanks supplied with UV or ozone-sterilized seawater; and
 - c. isolation of larvae and juvenile from broodfish
 - d. Vaccination of juveniles and non carrier status broodstock. This is a promising method of preventing VNN in groupers. Immunization of groupers with recombinant coat proteins prepared from RGNNV genotype strains induced virus-neutralizing antibodies that resulted in high protection against experimental infection of the virus. Moreover a multivalent vaccine reputedly providing total protection from infection by different genotypic variants of piscine nodavirus has been recently developed in the USA (US patent application #: **20080286294**). Reputedly this simple vaccine is suitable for administration to fish via the intramuscular or intraperitoneal route, or by bath and/or via the oral route.

Detailed description of eradication procedures for VNN and other highly-virulent infectious diseases of fish, as recommended by The World Organisation for Animal Health (OIE), is shown below (Table 13).

TABLE 13: Eradication protocols for VNN and other highly virulent -infective disease (Based on *OIE Manual of Diagnostic Tests for Aquatic animals* 2006) episodes

Introduction
Disinfection may be used for a range of reasons
1. In response to a major (catastrophic) disease episode or as a pre-emptive prophylactic sanitary practice within bio-security programmes designed to exclude or reduce the incidence of the disease
2. Total eradication of an infective disease to prevent its possible spread into the wild
The specific reason for disinfection, will determine the disinfection strategy used and how it is applied. When a notifiable disease such as VNN or an important but unlisted emerging disease occurs for the first time at a particular farm, at a particular site (i.e. at a quarantine facility), or within a region or state y previously believed to be free of that disease, it may be advisable, if not required, to eradicate the disease by depopulating the facility and performing a thorough disinfection of all or part of the facility. Following of the affected facility for a defined period of time may be warranted in some situations
Successful disease eradication protocols generally employ a number of the following disinfection methods in accordance with particular applications as discussed separately below
chlorine (as calcium hypochlorite, HTHT or a bleach solution containing a sufficient concentration of hypochlorite);
formaldehyde gas (from sublimated paraformaldehyde or concentrated formalin/potassium permanganate reaction);
iodine (as contained in iodophors);
lime (as calcium oxide or calcium hydroxide);
UV light (from natural sunlight);
ozone;
steam;
hot water (60°C);
concentrated acids;
desiccation;
detergents (for general cleaning, with some degree of disinfection capability for many products).

1. Disinfection of hatcheries and of broodstock rearing/holding facilities in response to a major (catastrophic) disease episode or as a pre-emptive prophylactic sanitary practice within bio-security programmes designed to exclude or reduce the incidence of the disease

Virtually all marine finish hatcheries and broodstock holding/rearing facilities use seawater that has been disinfected to remove potential pathogens, pests, and disease-carrying agents via mechanical filtration, UV irradiation, and/or chemical disinfection. This may be by passive source water filtration (i.e. by the use of seawater wells or well points) or by mechanical filtration using high pressure pumps and a variety of water filtration devices and pore sizes. Some facilities use filtration coupled with UV light disinfection of source water, while others use chemical disinfection methods, using either chlorination and de-chlorination or high doses of ozone and subsequent removal of residual oxidants. Chemical disinfection of source water typically requires the use of one or more water storage reservoirs in which the water is treated and detoxified before use in the hatchery or broodstock facility.

a) Disinfection of eggs and larvae

Vertically transmitted diseases, due to viruses such as VNN, and to bacterial and fungal disease agents, can be eliminated or have their incidence reduced through the routine use of disinfection protocols when used to surface disinfect eggs. A recommended method using Ozone on developing embryos is given above

b) Disinfection of tanks, equipment, pipes, air stones, etc.

For routine sanitation, hatchery and broodstock tanks (i.e. tanks for broodstock maturation, matting, spawning, larval rearing and indoor nursery) should be cleaned, disinfected and dried between use. Tanks used for the above-named purposes in hatcheries are typically precast fibreglass tanks or they are constructed of concrete coated or painted with resin-based coatings (e.g. epoxy or fiberglass resin) or lined with plastic liners manufactured for that purpose. After harvest of the stock from the tank, all loose objects and large-sized organic debris such as algae, faeces and left-over feed should be removed. With relatively small tanks, it is advisable after harvest of the stock to fill the tank to capacity, immerse all nonporous corrosion resistant equipment (i.e. airlines, air stones, stand pipes, screens, sampling containers, etc.) in the tank, and then add calcium hypochlorite to provide a minimum of 200 ppm of free chlorine. This should be allowed to set overnight. After the proper chlorinated soak-time, the tank can be drained and freshwater rinsed. Before draining the system, the treated water should be dechlorinated (see specific subsections on chlorination described below), unless appropriate effluent collection and treatment systems are in place. After the tank has been rinsed it should be allowed to completely dry. In the case of large tanks, an initial cleaning to remove loose debris should be followed by disinfection with a concentrated (~1600 ppm as chlorine) solution of calcium hypochlorite. All inside and outside surfaces should then be sprayed with this chlorine solution. The tank should then be allowed to set for several hours and then rinsed, filled and flushed. Surfaces should then be scrubbed free of all remaining material. After disinfection with chlorine, small or large tanks should be rinsed with clean water, then filled and flushed to ensure that no chlorine residues remain before the tank is restocked for another crop.

Disinfection of growout ponds

Following the routine harvest of a crop from a growout pond (or from a large tank or raceway used for growout of a crop), the pond (tank) bottom should be inspected. Large deposits of organic debris should be treated or removed. This is easily accomplished in lined tanks, raceways, or ponds (i.e. by flushing with a high pressure hose), but poses more of a challenge in large earth bottom ponds. Many methods of pond bottom disinfection and treatment between crops are practiced.

a) Chlorination

This disinfectant may be used for routine treatment of ponds between crops or when disease eradication is the goal. After draining the pond, remove (and dispose of [see section on carcass disposal in Section C.6]) as many animals from the system as is possible (this may be difficult in pond systems where the removal of large numbers of dead fish would not be practical). Partially refill the pond (or fill to capacity if required), discontinue the addition of new water, stop the discharge of effluent water, and remove any internal or external sources of aeration or aeration devices, which might be subject to corrosion. Then evenly distribute sufficient granulated calcium hypochlorite (such as Olin HTH) to provide a minimum residual free chlorine concentration of 10 ppm within the entire system's water. (NB: The person(s) applying the chlorine should wear waterproof outer wear to protect their skin, an approved chlorine mask, and goggles or a face shield for eye protection.) Redistribute additional calcium hypochlorite as often as required to maintain the residual concentration at near or 10 ppm. Allow the system to set for a minimum of 24-48 hours (especially if applied to large systems) at this minimal chlorine concentration. The chlorine will kill all fish and most, if not all, of the other organisms occupying the water column or resident in the pond. After the pond has been treated with chlorine for the required minimum time and before any water is discharged, neutralise the chlorine either passively by exposure to sunlight and air for approximately an additional 48 hours (without the addition of new chlorine) or by the addition of sodium thiosulphate at a rate of five (5) molecules of sodium thiosulphate for each four (4) molecules of chlorine (or the weight of sodium thiosulphate being 2.85 times the weight of chlorine in the water, see example table below).

Pond size	Average depth	Volume	Chlorine dose	Chlorine required	HTH (65% active Cl)	Thiosulphate required
1 hectare	1 m	10,000 m ³	10 ppm	100 kg	154 kg	285 kg

Periodic testing should be done for residual chlorine; water should not be discharged until it has reached 0 ppm (below detection level). Once the chlorine levels have been ascertained to be at 0 ppm, the system water can be safely dumped into the effluent system. In some culture systems, in particular raceways, tanks and small lined ponds (i.e. those systems in which the majority of the fish are not removed prior to disinfection), the dead fish should be collected for proper disposal (see section on carcass disposal in Section C.6).

b) Liming

The lime, as calcium oxide or calcium hydroxide, should be applied to a very moist bottom at a rate of 5000 kg/ha or 1500 kg/ha, respectively. Great care should be taken to assure that the lime is spread evenly over the soil surface. The pond should then be allowed to set for at least a week, or at least until the soil has dried to the point of cracking to a depth of approximately 10-20 cm. Additional lime may be applied after ploughing (see below) at a rate of 50% of that normally prescribed. The pond should again be dried for at least a week, depending on the weather.

c) Drying and ploughing

Whether or not a pond is treated by chlorination or liming or left to dry untreated, ploughing is a commonly used method of treating a pond bottom to reduce its organic content, improve nutrient recycling, buffer pH, eliminate pests, and achieve disinfection through a combination of microbial degradation, exposure to sunlight, aeration, and desiccation. In some regions, drying and ploughing of dry pond bottoms may only be possible during the 'dry season'. When pond drying is an option, the pond bottom should be allowed to dry until the surface has cracked to a depth of approximately 10 cm. Once this level of drying has been reached, the soil should be broken up to a depth of approximately 20 cm with a plough, tiller, disk harrow, tine harrow or other similar farm implement. Ponds treated in this manner should be left for at least a week before being refilled and restocked.

Disinfection of source water

Because VNN and a number of important diseases, can be introduced into hatcheries with source water when it contains vectors or carriers, biosecurity plans should include provisions for the disinfection of source water. This may be accomplished by a variety of means which may include one or some combination of the following strategies:

a) Filtration of source water - source water is pumped into a supply/settling canal where it first passes through coarse bar screens to remove large aquatic animals and debris. Then the water is passed through a series of progressively finer screens, and final filtration is accomplished by passing source water through a fine mesh (150-250 µm mesh size) bag screen before being introduced into a culture pond or storage reservoir.

b) Instead of using mesh nets, filtration structures can be placed in the supply canal system. A series of compartments within these structures are filled with filter matrixes, beginning with coarse gravel for initial removal of large aquatic animals and debris, an intermediate section which contains a finer matrix of sand and gravel, and the end section which contains fine sand.

c) Chlorination and de-chlorination - source water is pumped to a supply canal or directly into culture ponds or reservoirs (with or without filtration) and treated with sufficient chlorine to kill any potential vectors or carriers in the source water.

d) 'Zero' or reduced water exchange: Some fish hatcheries and farms use supplemental aeration and re-circulation of water in culture ponds and within the supply and discharge systems to reduce source water requirements. This reduces the volume of source water that must be disinfected before use, as well as reducing nutrient loss from farms with effluent.

Disease eradication and total facility clean-up

This action may be necessary for disease control when significant, untreatable diseases occur at sites where eradication is an option. The confirmed diagnosis of a notifiable diseases such as VNN, or of an important but unlisted emerging disease occurring for the first time at a particular facility at a particular site (i.e. at a quarantine facility), or within a state or region thereof previously believed to be free of that disease, are events wherein it may be advisable, if not required, to eradicate the disease by depopulating the affected facility and performing a thorough disinfection of all or part of the facility.

Following of the affected facility for a defined period of time may be warranted in some situations.

Total eradication of an infective disease agent to prevent its possible spread into the wild

The following steps/actions may be used to achieve eradication of a disease through a total facility clean-up (TCU):

a. Depopulate all living stocks from the affected facility

b. Discontinue stocking of the facility.

c. Harvest and sell (if permitted) marketable stocks through normal market channels.

For unmarketable stocks the following are options for disposable after harvest:

i) Incineration: burn collected shrimp in a government approved (if required) incinerator. The limitations to this procedure are inherent to the disposal of fish. That is, fish contain large amounts of water and therefore this procedure may only be feasible for small quantities of fish or to larger quantities if the fish have been dried prior to incineration.

Disinfection of culture tanks and ponds – as for general disinfection described above.

Clean-up procedures for facility components other than culture areas

In order for a TCU to be effective, it may be necessary to disinfect the entire facility after all the fish have either been harvested or disposed of in some other manner. After depopulation of the facility, every possible animate and inanimate carrier of the disease agent must be identified and either removed from the facility or thoroughly disinfected. The movement of disease agents between live fish or dead numerous fish can be easily understood, while the same can not be said for their movement via inanimate components. Hence, all areas, units, subunits or components which are contaminated or potentially contaminated must go through a cleaning and disinfection process.

a) Buildings

The disinfection regime used should be building-specific and dependent upon the use-pattern of that particular building.

i) Office buildings: these buildings would most often be subject only to foot traffic from people who have been in contaminated buildings or culture areas. Because of this, the greatest focus of attention should be the floors and cold storage units in the building. Floors should be thoroughly cleaned (if they are non-porous) with standard detergents and cleaning solutions, followed by a thorough drying. If the floors are carpeted, they should be vacuumed and cleaned with a detergent appropriate for carpets, or 'steam' cleaned. All other areas within these buildings, such as walls, bathrooms, desks, refrigerators, freezers, etc. should be examined for possibly contaminated materials (i.e. frozen shrimp in freezers) and any such item found and its container should be cleaned and disinfected or disposed of in a sanitary manner.

ii) Culture buildings: it must be assumed that these buildings have had direct contact with the disease agents and will therefore be handled in a different manner from that of the office buildings. The disinfection regime for these buildings will consist of two steps. First, the building should be thoroughly swept and/or vacuumed (where appropriate) to remove as much large-sized organic and inorganic debris as possible. This should be followed with the second step, treatment with chlorine. Chlorine solution (~1600 ppm) should be applied (by spraying) to all surfaces which are not prone to the corrosive actions of chlorine. Those surfaces which should not be chlorinated, can first be sponged with a iodophor solution minimally providing 200 ppm of free iodine. These can then be covered with plastic or any other protective material. Floor surfaces can be soak-chlorinated to a depth of 5 cm with a 200 ppm chlorine solution. This should be allowed to set for a minimum of hours. If many of the sprayed surfaces are somewhat susceptible to corrosion by chlorine, those surfaces can be freshwater-rinsed after the 48-hour treatment.

In buildings where disinfection with chlorine is not practical, fumigation with formaldehyde gas should be considered. After a general cleaning, fumigation of a sealable building can be initiated. The entire process, from the time the building is first gassed until it can be occupied again, should take a minimum of 36-60 hours. The entire building must be totally sealed off during the actual fumigation; there should be no means by which the gas can escape once it is placed in the building. If possible, the electrical service for the building should be turned off. The required environment for formaldehyde gas disinfection is a minimum temperature of 18°C with a high relative humidity (at saturation is best, i.e. floors should be wet, etc.). Generation of formaldehyde gas is accomplished by the addition of 17.5 g potassium permanganate to each 35 ml of 100% formalin (a 37-39% aqueous solution of formaldehyde gas) for each 2.83 m³ (100 ft³) of space. Ideally, each room in the structure should have its own source of formaldehyde gas to assure that all areas of the building are uniformly treated. The correct amount of each compound (potassium permanganate and formalin) should be weighed out into separate containers, the formalin should be placed in a non-plastic container that is at least 10 times the combined volume of both the formalin and the potassium permanganate.

(The person applying a formaldehyde gas fumigation should wear waterproof outer ware to protect their skin, an approved formaldehyde gas mask, and goggles or a face shield for eye protection.) The containers with the proper amounts of the two reagents should then be placed on the floor in the centre of the room, on a large disposable protective (plastic) mat. The formalin and potassium permanganate should not be mixed at this time. Once all rooms have the correct amounts of the two compounds, the building has been completely sealed and the environment modified as necessary, the actual fumigation can begin. The mixing of the two compounds must be done very rapidly and carefully as the reaction is

immediate and somewhat violent as formaldehyde gas is emitted. Starting with the room farthest from the exterior door, add the permanganate to the formalin and proceed to the next room. After all rooms have been completed, lock the exterior door and seal it from the outside with tape. The building should be allowed to set for a minimum of 12 hours. After this disinfection period the building should be flushed with clean air for 24-48 hours. There should be no detectable odour of formaldehyde when people are allowed to reoccupy the building.

An alternate method for the generation of formaldehyde gas is the sublimation of powdered paraformaldehyde. For each 2.83m³ (100 ft³) of space, approximately 28 g paraformaldehyde should be used. It can be sublimated by being placed in an electric fry pan, which has been set on high. This procedure is somewhat more dangerous, because formaldehyde is flammable and a spark from such a heating device could theoretically ignite the gas. The same procedures noted above for the formalin/permanganate mixture in regards to venting, etc. should also be followed for the use of paraformaldehyde.

iii) Processing buildings: these buildings are typically constructed to permit routine disinfection. For the most part, the procedures followed in the routine operation of such buildings are appropriate for a TCU, provided that the building, its cold rooms, and its freezers are also disinfected and thoroughly dried. If considered necessary, fumigation with formaldehyde gas may be done to insure destruction of the disease agent(s) of concern.

iv) Other buildings: buildings (feed storage, maintenance, tool rooms, etc.) should be treated somewhat like the office building. Care should be taken to remove all the large-sized debris, which would normally be found in relative abundance within these types of buildings. Potentially contaminated surfaces within such buildings should next be spray-chlorinated and allowed to set for 24-48 hours. This should be followed by a freshwater rinse. All equipment, which should not be exposed to the corrosive action of chlorine, should be removed before the spraying, and they should be disinfected by surface disinfection with 200 ppm of iodophor. Once the equipment has been disinfected, it can be brought back into the building. Fumigation with formaldehyde gas is another option for this type of building.

b) Culture support equipment and systems (not applicable to VNN until such time that infective pathways via algae and live feeds (rotifers and *Artemia*) have been demonstrated)

These are operational units of the culture facility which may be housed in a building.

i) *Artemia* systems: all *Artemia* decapsulation and cyst hatching units and tanks should be treated in the same manner as other tanks. They should be cleaned of all large debris, then filled to the top with clean water and calcium hypochlorite added to achieve a final concentration of 200 ppm (free Cl₂). Chlorination should be allowed to continue for 24-48 hours. The outside of such tanks may be spray-chlorinated (1600 ppm chlorine). Treated tanks can then be dechlorinated with sodium thiosulphate, drained, freshwater rinsed, and allowed to dry for a minimum of one week. Unopened containers of *Artemia* cysts at the facility can be retained. These should, however, be surface disinfected with chlorine (200 ppm) or iodophor (200 ppm).

ii) Algae systems: containers, tanks, incubators and rooms used to produce algae for feeding the larval stages of fish may be handled and disinfected in nearly the same way as other tanks systems. The only major difference being that special care must be taken to assure that all chlorine residues have been rinsed from the units before they are used again. In the case of the culture tubes, flasks, carboys, and flasks used to culture algae, a combination of acid (10% HCl) rinse or steam sterilisation can be used in lieu of disinfection with chlorine or iodophor.

Disinfection of stock cultures of living algae is not possible. The use of disinfection is clearly out of the question; any compound which would kill the disease agent would likewise kill the algae. Hence, there are two basic methods of minimising the chance of a disease agent being present in the stock cultures.

Dilution: all stock cultures can be cloned from the existing stocks. Each culture should be diluted either by means of serial dilutions (for broth cultures) or streaked for single colonies (agar cultures). All dilutions must be performed using strict aseptic techniques with all media being properly autoclaved. Passages from the stock cultures should not occur until the algae culture room has itself been disinfected as per the above building procedures. Once a culture has been diluted and cloned by either of these methods, to the point where there remains only one cell of the original culture, the risk is negligible that a fish disease agent may be present.

New Stock Cultures: If existing stock culture are discarded in a TCU, new stocks should be purchased from algae supply laboratories, or obtained from other sources where contamination with (shrimp) disease agents is unlikely, such as isolating desired species from wild populations of algae. New stock cultures should not be obtained from any facility that also cultures fish and may be contaminated with fish disease agents of concern.

iii) Farm equipment: nets, seines, porous air-line tubing, etc. which are relatively inexpensive and easily obtainable should be discarded and removed from the facility during a TCU rather than being disinfected as they are not readily disinfected and chlorine is likely to damage them and shorten their useful life.

Non-expendable equipment such as large size flexible plastic tubing, pumps and pipes, transfer tanks, cages, harvest cages, harvest tables, Secchi disks, laboratory glassware, etc. should be soak-chlorinated in 200 ppm solutions for 24-48 hours. This is most easily accomplished by placing these objects in the tanks that are filled with 200 ppm solutions of chlorine. Care should be taken to have all items completely submerged (use heavy items to weigh-down more buoyant objects). A good guide is to place everything (except those that are to be thrown away) that is loose or can be unsecured from its point of attachment, into the 200 ppm chlorine solution in their respective tanks.

In the case of those similar type items which are associated with ponds, they should be placed in a special series of tanks set up near their respective ponds. These tanks should be filled with 200 ppm chlorine solutions. Following soak-chlorination, these items should be allowed to dry and be exposed to natural UV (sunlight) sterilisation. They should be turned at least once to expose all areas of the items to direct sunlight.

Tools and machinery, such as tractors, trucks, portable and stationary power tools, etc., should be thoroughly cleaned with standard cleaning solutions. All traces of mud, feed, etc. must be removed from these items. Following this, disinfection of surfaces likely to have been contaminated in normal use should be rinsed off with an iodophor solution (at a concentration of 200 & nbsp;ppm) or cleaned with steam.

Small tools and instruments such as, scales and balances, test instruments, small power tools, etc., should be gently sponged off with 200 ppm of chlorine solutions if they are inert plastic or 200 ppm of iodophor if they are otherwise. These should then be placed back in their respective buildings during the formaldehyde fumigation. High precision electronic test equipment should not be subjected to the fumigation, especially if there has been little chance that it was ever contaminated.

iv) 'New-Water' Plumbing: all new-water plumbing which is contained within buildings, especially those which have blind ends or terminate in manifolds, should be filled with a minimum 200 ppm chlorine solution. The chlorine solution should be held in the lines for 24-48 hours minimum, followed by clean water rinsing. Pipes may also be disinfected by recirculating hot water (>60°C) through them for several hours.

v) Uniforms, boots, etc.: all items worn or used by employees should be either disposed of or thoroughly washed and disinfected. In the case of clothing, such as coveralls, normal washing which incorporates a chlorine bleach is sufficient, especially if accompanied by sun drying. Other items, such as boots, gloves and other non-cloth items can be safely soak-chlorinated in a 200 ppm chlorine solution. This should be followed by a freshwater rinse. These items should also be contained within their respective buildings during formaldehyde fumigation.

vi) Feed items: all feed items, such as prepared feeds, fresh feeds should be removed from the facility and replaced with new feeds from sources known to be free of contamination by disease-causing agents.

Re-stocking of disinfected farms

Following a TCU, restocking of the disinfected facilities or farms should be accomplished only with stocks known to be free of VNN and other notifiable or other emerging or significant fish diseases of concern.