Diseases of cultured marine fishes caused by Platyhelminthes (Monogenea, Digenea, Cestoda)

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SUMMARY

Mariculture is a rapidly developing industrial sector. Generally, fish are maintained in net cages with high density. Cage culture systems allow uncontrolled flow of sea water containing potentially infectious stages of fish parasites. In such culture conditions, prevention of such parasitic infections is difficult for parasites with life cycles that complete within culture sites, among which monogeneans and blood flukes are the most important platyhelminthes. Intense monogenean infections induce respiratory and osmo-regulatory dysfunctions. A variety of control measures have been developed, including freshwater bath treatment and chemotherapy. The potential to control monogenean infections through selective breeding, modified culture techniques to avoid infection, and general fish health management are discussed. It should be noted that mariculture conditions have provided some host-specific monogeneans with a chance to expand their host ranges. Blood flukes sometimes induce mass mortality among farmed fish. In-feed administration of praziquantel is the best solution to treat infected fish. Some cases are described that show how international trade in marine fish has resulted in the spread of hitherto unknown parasites into indigenous farmed and wild fish.

Key words: mariculture, Platyhelminthes, Monogenea, Digenea, disease.

INTRODUCTION

Based on the latest statistics, the worldwide catch of marine fish was 8·35 million tons in 2011, only a 0·16% increase from 10 years ago (FAO, 2013). On the other hand, production of cultured marine fish is rapidly increasing, with production being 3·89 million tons in 2011, a 69·5% increase from 10 years ago (FAO, 2013). Among them, salmonid culture in Europe, North America and Chile comprises 55·9% of the total mariculture production, followed by those of carangids (0·28 million tons or 7·2% of the total production), sparids (0·26 million tons; 6·6%) and percichthyids and moronids (0·24 million tons; 6·2%). With respect to helminth parasitic diseases of cultured marine fishes, few serious helminth infections are known to occur among maricultured salmonids, whereas carangid, sparid, percichthyid and serranid fishes have important helminth diseases.

Traditionally marine fish were cultured in coastal ponds and natural coastal enclosures. These culture systems were practiced on a small scale, compared with the large-scale floating net cage system which appeared around 1960, which was first adopted for culture of Japanese amberjack *Seriola quinqueradiata* (Eng and Tec, 2002). Typically, cages are cubic with one side of 10 m or smaller in size. This system spread rapidly in western Japan, as it has many advantages over the traditional methods in terms of water exchange, cost for setup and maintenance, management of fishes in cages and applicability to other marine fish species. Recently, new types of larger net cages and offshore cages for bluefin tunas and submerged cages in rough areas have appeared (Beveridge, 2002). Net cages are not suitable for some fishes. Flat fishes like bastard halibut, *Paralichthys olivaceus*, are mainly cultured in land-based tanks with flow-through sea water.

In mariculture, fish are introduced into farm sites in the form of culture seed, either wild-caught or artificially produced. With the progress of artificial seed production techniques, the latter type is used more frequently. The former type is still the main source of seed for Japanese amberjack and greater amberjack, *Seriola dumerili*, because juveniles are caught in large numbers without any sign of over-fishing and generally wild-caught juveniles have fewer health problems.

Traditionally marine fish are fed with chopped or minced raw trash fish. However, with expanding culture scales, this type of feeding caused serious environmental pollution problems now largely resolved by replacing it with moist pellets and dry extruded pellets.

Platyhelminth parasites, comprising monogeneans, digeneans and cestodes, are quite common in maricultured fish. They usually infect feral fish in low numbers, causing little pathology. However, mariculture farms, where fish are maintained in high densities, create favourable conditions for parasite proliferation because of the increased probability of encountering a host. Culture systems also impose considerable stress on farmed fish, which generally

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increases their susceptibility to parasitic infections. In spite of its many advantages as mentioned above, cage culture is an open system, where parasites are transmitted easily among fish in cages through free exchange of water. Some monogeneans are especially harmful in cage culture, as eggs entangle culture nets with their filamentous appendages, making fish in cages easy targets for re-infection. Blood flukes are dangerous parasites of vertebrates including teleost fish. Considerable numbers of fish blood flukes are known to infect farmed marine fish. Their intermediate hosts have rarely been identified, but they are present in and around many mariculture farm areas. Consequently, with rapid expansion and intensification of mariculture, economic losses caused by platyhelminthes have increased dramatically. Damage to mariculture industries ranges from mortality of farmed fish to growth retardation and sometimes loss of market value due to the unaesthetic appearance of infected fish.

Aquarium fish are not included here. Common names are based on FishBase (Froese and Pauly, 2014).

MONOGENEA

Monogeneans (Platyhelminthes: Monogenea) are hermaphrodites and mostly ectoparasites of fish, infecting the host's outer surfaces including the gills, skin and fins and less commonly buccal, branchial and nasal cavity linings. Except for the viviparous gyrodactylids, monogeneans are oviparous and typically, hatching larvae, or oncomiracidia, can swim in the water. Upon encounter with a suitable host, they either attach directly to the host surfaces or invade by inflowing currents to the gills, and start to grow. Between 4000 and 5000 species of monogeneans are currently described (Whittington and Chisholm, 2008). However, the actual number of species is much higher, as so many fish, including cultured ones, have not yet been fully examined for this group of parasites. Important monogeneans of maricultured fish are listed in Table 1.

Monogeneans comprise the Monopisthocotylea and Polyopisthocotylea. Monopisthocotyleans are generally small, mostly up to 1 cm long. Most members of the families Dactylogyridae, Ancyrocephalidae, Diplectanidae and Gyrodactylidae are less than 1 mm long, and so are difficult to detect without the aid of a stereomicroscope. Polyopisthocotyleans are generally larger than monopisthocotyleans and some grow over 3 cm long. From species to species, clamps which grasp or suck gill tissues for attachment vary in shape and number (from eight to more than 100). In some polyopisthocotyleans, the body is asymmetrical due to the number and size of clamps being unequal between the two rows. Polyopisthocotyleans have a large uterus which can hold a large number of eggs.

Biology

Monopisthocotyleans basically feed on epidermis, whereas polyopisthocotyleans feed exclusively on host blood. In most cases monogeneans show a high degree of host specificity. However, some monogeneans show almost no host specificity, like the capsalids *Neobenedenia melleni*, having been recovered from more than 100 species of fish worldwide (Whittington and Chisholm, 2008) and *Neobenedenia girellae* (a synonym of *N. melleni* according to Whittington and Horton, 1996) and *Benedenia epinepheli* from 15 and seven species, respectively, of farmed fish in Japan (Ogawa *et al*. 1995*a*, *b*). This does not mean that all fish species show the same degree of susceptibility to these monogeneans. For example, susceptibility against *N. girellae* infection differed among greater amberjack, Japanese amberjack and bastard halibut (Ohno *et al*. 2008). When these three species of fish with approximately the same body size were exposed to *N. girellae* oncomiracidia in a tank, the parasite infected more intensely and grew faster in greater amberjack than in the other two fish species.

Biological parameters of monogeneans, such as generation time, fecundity and longevity, have been poorly studied or are completely unknown (Whittington and Chisholm, 2008). Farmed fish may provide a better source of information on monogenean biology than wild fish, as we can make a regular monitoring of infection under controlled conditions. Still, data obtained from farmed fish can be biased compared with those from wild fish, as they are kept under different conditions. Table 2 summarizes some biological data of monogeneans infecting marine fish cultured commercially or experimentally, which show variance with parasite species, host responses, water temperature (WT) and culture conditions. Most of the data in Table 2 are based on experiments where fish were kept in aquaria and exposed to oncomiracidia.

Concerning the generation time (time required for maturation on host fish), the monopisthocotyleans *Anoplodiscus cirrusspiralis* (Anoplodiscidae), *Diplectanum aequans* (Diplectanidae), *Benedenia seriolae* and *N. girellae* have a shorter pre-patent period of 2–4 weeks at 20–25 °C (Kearn *et al*. 1992; Bondad-Reantaso *et al*. 1995*a*; Cecchini *et al*. 1998; West and Roubal, 1998*a*; Tubbs *et al*. 2005; Lackenby *et al*. 2007; Hirayama *et al*. 2009) than the polyopisthocotyleans *Zeuxapta seriolae* (Heteraxinidae), *Heterobothrium okamotoi* (Diclidophoridae) and *Neoheterobothrium hirame* (Diclidophoridae) of 4–7 weeks in the same range of WT (Ogawa and Inouye, 1997; Tsutsumi *et al*. 2003; Tubbs *et al*. 2005) (Table 2).

The lifespan is largely affected by WT, but no fundamental difference is seen between monopisthocotyleans and polyopisthocotyleans (Table 2).

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Table 1. Platyhelminthes causing problems in mariculture

	Parasite families	Parasites (scientific names)	Hosts (scientific names)	References
Monogenea (Monopisthocotylea)	Capsalidae	*Benedenia epinepheli*	*Epinephelus* spp., etc.	Ogawa *et al*. (1995*a*)
	Capsalidae	*Benedenia seriolae*	*Seriola* spp.	Okabe (2000); Sharp *et al*. (2004); Ernst *et al*. (2005); Kinami *et al*. (2005); Williams *et al*. (2007); Mooney *et al*. (2008); Nagakura *et al*. (2010); Hirazawa *et al*. (2013); Ozaki *et al*. (2013)
	Capsalidae	*Neobenedenia* spp.	>100 fish species	Ogawa *et al*. (1995*b*, 2006); Umeda and Hirazawa (2004); Kinami *et al*. (2005); Ohashi *et al*. (2007*a*, *b*); Whittington and Chisholm (2008); Hirazawa *et al*. (2013); Militz *et al*. (2013); Shirakashi *et al*. (2013*a*, *b*)
	Diplectanidae	*Diplectanum aequans*	*Dicentrarchis labrax*	González-Lanza *et al*. (1991); Dezfuli *et al*. (2007)
	Diplectanidae	*Diplectanum laubieri*	*Dicentrarchis labrax*	González-Lanza *et al*. (1991)
	Anoplodiscidae	*Anoplodiscus cirrusspialis*	*Chrysophrys auratus*	West and Roubal (1998*a*, *b*)
Monogenea (Polyopisthocotylea)	Microcotylidae	*Bivagina tai*	*Chrysophrys major*	Ogawa (1988)
	Microcotylidae	*Microcotyle sebastis*	*Sebastes schlegeli*	Kim and Choi (1998); Kim and Cho (2000); Kim *et al*. (1998, 2000, 2001)
	Microcotylidae	*Sparicotyle chrysophrii*	*Sparus aurata, Diplodus puntazzo*	Mladineo and Maršić-Lučić (2007); Mladineo *et al*. (2009); Sitjà-Bobadilla *et al*. (2006); Antonelli *et al*. (2010)
	Heteraxinidae	*Zeuxapta seriolae*	*Seriola lalandi*	Sharp *et al*. (2004); Mansell *et al*. (2005); Williams *et al*. (2007)
	Heteraxinidae	*Heteraxine heterocerca*	*Seriola quinqueradiata*	Mooney *et al*. (2008)
	Diclidophoridae	*Heterobothrium okamotoi*	*Takifugu rubripes*	Ogawa and Inouye (1997); Ogawa (2002, 2012); Yamabata *et al*. (2004); Nakane *et al*. (2005); Kimura *et al*. (2009)
	Diclidophoridae	*Neoheterobothrium hirame*	*Paralichthys olivaceus*	Anshary *et al*. (2001, 2002); Yoshinaga *et al*. (2009)
Digenea	Galactosomidae	*Galactosomum* sp.	*Seriola quinqueradiata, Takifugu rubripes, Oplegnathus fasciatus*	Kimura and Endo (1979); Yasunaga *et al*. (1981)
	Acanthocolpidae	*Stephanostomum tenue*	*Oncorhynchus mykiss*	McGladdery *et al*. (1990)
	Aporocotylidae	*Cardicola aurata*	*Sparus aurata*	Holzer *et al*. (2008)
	Aporocotylidae	*Cardicola orientalis*	*Thunnus orientalis*	Shirakashi *et al*. (2012*a*)
	Aporocotylidae	*Cardicola opisthorchis*	*Thunnus orientalis*	Shirakashi *et al*. (2012*a*, *b*); Sugihara *et al*. (2014)
	Aporocotylidae	*Cardicola forsteri*	*Thunnus maccoyii*	Aiken *et al*. (2006, 2008); Cribb *et al*. (2011); Kirchhoff *et al*. (2011); Hardy-Smith *et al*. (2012)
	Aporocotylidae	*Paradeontacylix balearicus*	*Seriola dumerili*	Crespo *et al*. (1992); Repullés-Albelda *et al*. (2008)
	Aporocotylidae	*Paradeontacylix grandispinus*	*Seriola dumerili*	Ogawa *et al*. (1989, 1993); Ogawa and Fukudome (1994)
	Aporocotylidae	*Paradeontacylix kampachi*	*Seriola dumerili*	Ogawa *et al*. (1989, 1993); Ogawa and Fukudome (1994)
	Aporocotylidae	*Psettarium* spp.	*Takifugu rubripes*	Ogawa *et al*. (2007)
Cestoda	Gilquinidae	*Gilquinia squali*	*Oncorhynchus tshawytscha*	Kent *et al*. (1991)
	Lacistorhynchidae	*Protogrillotia zerbiae*	*Seriola dumerili*	Ogawa *et al*. (2012)

Table 2. Biological data on monogeneans from marine fish in culture conditions

Parasite name	Data	WT	Host fish	Culture condition	References
Time required for maturation					
Anoplodiscus cirrusspiralis	20 days	15·2–23·3 °C	*Chrysophrys auratus*	aquarium	West and Roubal (1998*a*)
Diplectanum aequans	35 days	15·5 °C	*Dicentrarchus labrax*	aquarium	Cecchini *et al*. (1998)
Diplectanum aequans	25 days	20 °C	*Dicentrarchus labrax*	aquarium	Cecchini *et al*. (1998)
Diplectanum aequans	15 days	26 °C	*Dicentrarchus labrax*	aquarium	Cecchini *et al*. (1998)
Diplectanum aequans	9 days	30 °C	*Dicentrarchus labrax*	aquarium	Cecchini *et al*. (1998)
Benedenia seriolae	14 days	22 °C	*Seriola lalandi*	aquarium	Kearn *et al*. (1992)
Benedenia seriolae	48 days	13 °C	*Seriola lalandi*	aquarium	Tubbs *et al*. (2005)
Benedenia seriolae	25 days	18 °C	*Seriola lalandi*	aquarium	Tubbs *et al*. (2005)
Benedenia seriolae	20 days	21 °C	*Seriola lalandi*	aquarium	Tubbs *et al*. (2005)
Benedenia seriolae	41 days	14 °C	*Seriola lalandi*	aquarium	Lackenby *et al*. (2007)
Benedenia seriolae	24 days	18 °C	*Seriola lalandi*	aquarium	Lackenby *et al*. (2007)
Benedenia seriolae	16 days	22 °C	*Seriola lalandi*	aquarium	Lackenby *et al*. (2007)
Benedenia seriolae	14 days	26 ± 0·5 °C	*Seriola lalandi*	aquarium	Lackenby *et al*. (2007)
Neobenedenia girellae	10–11 days	25 °C	*Paralichthys olivaceus*	aquarium	Bondad-Reantaso *et al*. (1995*a*)
Neobenedenia girellae	9 days	25·5 ± 0·5 °C	*Seriola dumerili*	aquarium	Hirayama *et al*. (2009)
Zeuxapta seriolae	> 52 days	13 °C	*Seriola lalandi*	aquarium	Tubbs *et al*. (2005)
Zeuxapta seriolae	37 days	18 °C	*Seriola lalandi*	aquarium	Tubbs *et al*. (2005)
Zeuxapta seriolae	25 days	21 °C	*Seriola lalandi*	aquarium	Tubbs *et al*. (2005)
Heterobothrium okamotoi	about 7 weeks	16·8–26·8 °C	*Takifugu rubripes*	aquarium	Ogawa and Inouye (1997)
Neoheterobothrium hirame	52–66 days	15 °C	*Paralichthys olivaceus*	aquarium	Tsutsumi *et al*. (2003)
Neoheterobothrium hirame	31–45 days	20 °C	*Paralichthys olivaceus*	aquarium	
Neoheterobothrium hirame	24–31 days	25 °C	*Paralichthys olivaceus*	aquarium	
Longevity					
Anoplodiscus cirrusspiralis	at least 6 months (median 1.5 months)	15·2–23·3 °C	*Chrysophrys auratus*	aquarium	West and Roubal (1998*a*)
Anoplodiscus cirrusspiralis	2–20 weeks (mean 9·12 weeks)	14·5–24·8 °C	*Chrysophrys auratus*	cages in on-shore pool	West and Roubal (1998*b*)
Bivagina tai	3–5 months in winter and 2–3 months in spring	10–28 °C	*Chrysophrys major*	sea cages	Ogawa (1988)
Heterobothrium okamotoi	within 4 months	16·8–26·8 °C	*Takifugu rubripes*	aquarium	Ogawa and Inouye (1997)
Neoheterobothrium hirame	up to 122 days	15 °C	*Paralichthys olivaceus*	aquarium	Tsutsumi *et al*. (2003)
Neoheterobothrium hirame	up to 62 days	20 °C	*Paralichthys olivaceus*	aquarium	Tsutsumi *et al*. (2003)
Neoheterobothrium hirame	up to 52 days	25 °C	*Paralichthys olivaceus*	aquarium	Tsutsumi *et al*. (2003)
Fecundity					
Anoplodiscus cirrusspiralis	in excess of 3000 eggs in lifetime	15·2–23·3 °C	*Chrysophrys auratus*	aquarium	West and Roubal (1998*a*)
Benedenia seriolae	859–2388 eggs (mean: 1398 eggs) per day	23·8 °C	*Seriola quinqueradiata*	aquarium	Mooney *et al*. (2008)
Heteraxine heterocerca	218–1148 eggs (mean: 404 eggs) per day	”	*Seriola quinqueradiata*	aquarium	Mooney *et al*. (2008)
Heterobothrium okamotoi	96 ± S.D. of 117 eggs per day	10 °C	*Takifugu rubripes*	aquarium	Yamabata *et al*. (2004)

Table 2. (*Cont.*)

Parasite name	Data	WT	Host fish	Culture condition	References
Heterobothrium okamotoi	142 ± 119 eggs per day	15 °C	*Takifugu rubripes*	aquarium	Yamabata *et al.* (2004)
Heterobothrium okamotoi	307 ± 168 eggs per day; 209 ± 138 eggs per day	20 °C	*Takifugu rubripes*	aquarium	Yamabata *et al.* (2004)
Heterobothrium okamotoi	454 ± 279 eggs per day; up to 32 000 eggs in lifetime	25 °C	*Takifugu rubripes*	aquarium	Yamabata *et al.* (2004)
Heterobothrium okamotoi	301 ± 262 eggs per day	30 °C	*Takifugu rubripes*	aquarium	Yamabata *et al.* (2004)
Neoheterobothrium hirame	203 eggs per day	10 °C	*Paralichthys olivaceus*	aquarium	Tsutsumi *et al.* (2002)
Neoheterobothrium hirame	578 eggs per day	15 °C	*Paralichthys olivaceus*	aquarium	Tsutsumi *et al.* (2002)
Neoheterobothrium hirame	781 eggs per day	20 °C	*Paralichthys olivaceus*	aquarium	Tsutsumi *et al.* (2002)
Neoheterobothrium hirame	651 eggs per day	25 °C	*Paralichthys olivaceus*	aquarium	Tsutsumi *et al.* (2002)

* : estimated number based on daily egg output X duration of adult stage.

Longevity of *Bivagina tai* (Microcotylidae) was estimated from a 2-year field survey of a single population of red seabream, *Chrysophrys major* (as *Pagrus major*) maintained in an experimental net cage, in which the number of clamps was used as an indicator of parasite age (Ogawa, 1988). It should be noted that *A. cirrusspiralis* on susceptible fish lived longer that those on resistant fish (West and Roubal, 1998*b*).

Water temperature and experimental conditions aside, fecundity of monogeneans varies widely. For example, *A. cirrusspiralis* has a potential to deposit in excess of 3000 eggs throughout its lifespan (West and Roubal, 1998*a*), whereas *B. seriolae* produced a mean value of 1398 eggs in one day (Mooney *et al.* 2008), suggesting a much higher fecundity by the latter parasite. Only a few data are available on the lifetime egg output by monogeneans. *Heterobothrium okamotoi* produced an average of 454 eggs per day at 25 °C (Yamabata *et al.* 2004). Considering its generation time, longevity and a daily egg output, a single *H. okamotoi* could lay up to 32 000 eggs at 25 °C. At high WT, monogeneans produce more eggs but have shorter lifespans than at low WT. However, no data are available for any fish monogenean on how different WTs affect the lifetime egg production. According to the same method of calculation as that used for *H. okamotoi* above, the lifetime egg production by *N. hirame* was estimated to be up to 40 000 eggs at 15 °C, 24 000 eggs at 20 °C and 18 000 eggs at 25 °C, the number being negatively correlated with WT (Tsutsumi *et al.* 2002, 2003).

Monogeneans of farmed marine fish often show seasonality in infection. Generally, high WT induces high reproductive potential for parasites. However, unexpectedly, high levels of infection were also experienced at low WT. For example, prevalence of infection of the monopisthocotyleans *D. aequans* and *Diplectanum laubieri* on the gills of European seabass *Dicentrarchus labrax* and the polyopisthocotylean *Sparicotyle chrysophrii* (Microcotylidae) on the gills of gilthead sea bream *Sparus aurata* were high in winter (González-Lanza *et al.* 1991; Antonelli *et al.* 2010). A monthly monitoring of *B. tai* infection of red seabream cultured in a single net cage for 2 years showed infection peaks in early summer and winter, with WT ranging from 10 to 28 °C (Ogawa, 1988). The 0-year-old seabream had the highest level of infection in winter, while infection was modest in the following winter, suggesting the peak infection resulted from lowered resistance of the small fish to infection at low temperatures. WT was a major factor regulating these seasonal fluctuations, affecting both the reproductive potential of the parasites and resistance to infection by the host fish.

A mixed infection of *B. seriolae* and *N. girellae* sharing the same habitat on greater amberjack was monitored for 1 year (WT between 20 and 29 °C) (Kinami *et al.* 2005). *Neobenedenia girellae* was

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dominant in October to February. With increasing WT, *B. seriolae* appeared and its ratio to *N. girellae* increased up to above 90% in June. Then, in July, *N. girellae* began to increase again and almost replaced *B. seriolae* in September. It appears that the observed seasonal fluctuation was affected by different optimal temperatures for reproduction of each parasite and possibly by competition between the two parasites.

Pathogenicity and associated host responses

Mass mortalities associated with monogenean infection sometimes occur among maricultured fish. For example, 100% mortality was recorded in *N. girellae* infection of greater amberjack cultured in Okinawa, Japan (Ogawa *et al*. 1995*a*). Mass mortality of yellowtail amberjack heavily infected with *Z. seriolae* cultured in cages was reported in the Mediterranean (Grau *et al*. 2003).

Monopisthocotyleans including Capsalidae, Diplectanidae, Anoplodiscidae, Ancyrocephalidae and Gyrodactylidae are known to be harmful to maricultured fish. Pathological changes include excess secretion of mucus, haemorrhage, tissue loss due to feeding activities and inflammatory reactions to parasite attachment such as hyperplasia around their attachment organs (Buchmann and Bresciani, 2006; Whittington and Chisholm, 2008). In *D. aequans* infection on the gills of European seabass, the attachment sites were marked by haemorrhages and a white mucoid exudate. Hyperplasia of the epithelium and inflammatory and haemorrhagic foci, especially around the areas of parasite attachment were observed (Fig. 2A). The opisthaptor penetrates deeply into the gills, inducing disruption and fusion of the secondary lamellae (González-Lanza *et al*. 1991; Dezfuli *et al*. 2007).

Capsalids generally cause considerable damage to the host skin through the attachment by the haptor and feeding activity. An infection experiment in a small aquarium demonstrated that *N. girellae* infection affected growth of greater amberjack with a parasite density of more than $0{\cdot}285 \pm 0{\cdot}042/cm^2$ fish surface (Hirayama *et al*. 2009). The epidermis of infected fish became thinner as infection was prolonged. Furthermore, in *B. seriolae* and *N. girellae* infection of cultured *Seriola* spp., skin lesions such as ulcers and scale loss formed by the feeding and attaching activities of the parasites deteriorate after the fish rub their body against net cages to get rid of the parasites (Fig. 1). *Neobenedenia girellae* tended to gather on the eyes of cobia *Rachycentron canadum* cultured in Taiwan (Ogawa *et al*. 2006). The epithelial layer of the cornea was often partially lost, and the collagenous stroma was thickened, oedematous and associated with massive inflammatory cell infiltration (Ogawa *et al*. 2006).

Fig. 1. Extensive haemorrhage on the skin and ragged tail fin of greater amberjack, *Seriola dumerili*, caused by *N. girellae* infection (top) and rubbing its body to get rid of the parasite that followed (bottom) (photos kindly provided by Dr Sho Shirakashi).

Fish heavily infected with polyopisthocotyleans such as Microcotylidae, Heteraxinidae and Diclidophoridae show emaciation, dark body colour, slow swimming and anorexia (Ogawa, 2002). These non-specific disease signs are mainly caused by anaemia (Fig. 2B). Negligible pathological changes are usually associated in the attachment sites (Fig. 2C). However, in *Z. seriolae* infection of yellowtail amberjack *Seriola lalandi*, hyperplasia and fusion of gill lamellae were observed away from the parasite attachment sites (Mansell *et al*. 2005). It was speculated that those changes were caused by feeding activity of the parasite. *Heterobothrium okamotoi* on Japanese pufferfish also causes anaemia and severe pathological changes in the branchial cavity wall. Severe epithelial hyperplasia and infiltration of inflammatory cells into the dermis and subcutaneous tissue were observed. As a result, its haptor and posterior body became embedded in host tissues (Fig. 2D, E). The epithelial lining around the parasite is degenerative and discontinuous due to actions generated by the clamps, leading to invasion of seawater around the parasite. The tissue surrounding the parasite is necrotic, giving off a putrid smell (Ogawa, 2002). *Neoheterobothrium hirame* infects the buccal cavity wall of bastard halibut, with its posterior body and haptor embedded in host tissue, inducing significant inflammation and hyperplasia at the parasite attachment site (Anshary and Ogawa, 2001). Leucocytes constituting monocytes/macrophages, granulocytes and dense granular cells infiltrated and adhered to the parasite tegument (Nakayasu *et al*. 2003). The tegument was partially disrupted and phagocytized by infiltrating host cells, leading to the death and elimination of the parasite (Nakayasu *et al*. 2005).

Generally, fish develop little or no immunity against ectoparasites. However, innate and acquired protection against monogenean infection have

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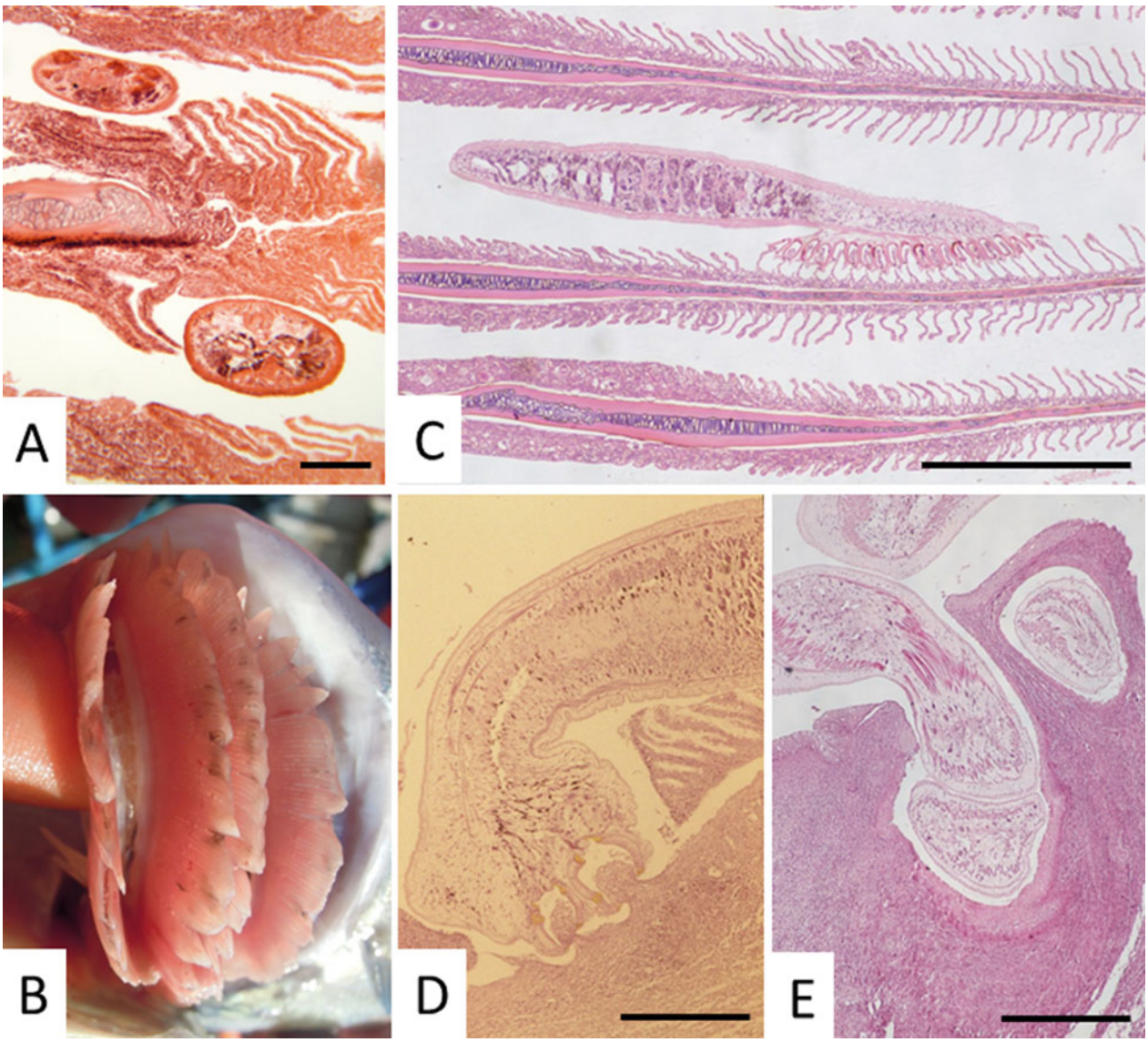

Fig. 2. Pathogenicity of monogeneans. (A) anaemia caused by heavy *Zeuxapta* infection of greater amberjack, *Seriola dumerili*; (B) histological section of the gill of greater amberjack infected with *Zeuxapta japonica* (scale: 0·5 mm); (C) gills of European seabass, *Dicentrarchus labrax* infected with *Diplectanum aequans* (scale: 0·1 mm) (photo kindly provided by Dr Ariadna Sitjà-Bobadilla); (D) skin of the branchial cavity wall of Japanese pufferfish, *Takifugu rubripes* pinched by clamps of *Heterobothrium okamotoi* (scale: 0·5 mm); E: advanced pressure atrophy of host tissues caused by *H. okamotoi* in Japanese pufferfish (scale: 0·5 mm).

been suggested in many cases. Most studies on the mechanisms that underlie host reactions have been carried out in freshwater fish (see Buchmann and Bresciani, 2006). Studies on the immune responses against monogenean infections of farmed marine fish are fragmentary, as discussed below.

Populations of silver seabream showed different degrees of innate resistance against the monogenean *A. cirrusspiralis* (West and Roubal, 1998*b*). Fewer than 50% of oncomiracidia that attached successfully reached maturity. In spite of such innate resistance, intense infection did not confer subsequent protection (West and Roubal, 1998*b*). Lectins may act as an innate element of resistance. A novel mannose-specific lectin named as pufflectin was detected in epithelial cells in the skin, gills and oral cavity of Japanese pufferfish (Tsutsui *et al*. 2003), which binds to *H. okamotoi* under *in vitro* conditions (Tsutsui *et al*. 2005). This lectin may be involved in the innate protection against *H. okamotoi*.

There are several reports that marine fish infected with monogeneans acquire resistance or immunity against re-infection. Primed bastard halibut, which had been previously infected with *N. girellae* and treated by freshwater bath, had lower intensities of infection and smaller body size of parasites in secondary infections, compared with those of naïve control fish (Bondad-Reantaso *et al*. 1995*b*). Alternatively, such apparent acquired resistance may actually be resource depletion and subsequent parasite decline (Lindenstøm and Buchmann, 2000; Hirayama *et al*. 2009). However, it seemed that

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antibodies against the monogenean were not involved in this reaction, as the level of antibody in the sera of primed fish was not raised. When fish were immunized by injection with sonicated parasite antigen, there was no significant difference in the parasite counts between antigen-injected fish and PBS-injected control after challenge with *N. girellae* oncomiracidia. These results indicate that the protection induced by the previous infection was not associated with the humoral antibody (Bondad-Reantaso *et al*. 1995*b*).

Kim *et al*. (2000) reported a different kind of host immune response against monogenean infection. They suggested that Korean rockfish, *Sebastes schlegeli*, can acquire some degree of immunity against *Microcotyle sebastis* (Microcotylidae) infection through specific and non-specific immune stimuli. Rockfish injected with homogenized parasite antigen emulsified in an equal volume of Freund's complete adjuvant (FCA) or with FCA had significantly lower intensities of infection than controls which received PBS injection after challenge with parasite eggs (Kim *et al*. 2000).

Farmed Japanese pufferfish persistently infected with *H. okamotoi* for longer than 1 year developed protection against infection. Infection experiments suggest they showed resistance against re-infection on the following three occasions: settlement of oncomiracidia on gills, early developmental stages when the mode of attachment changes from hooks to clamps, and migration of immature worms from the gills to the branchial cavity wall (Nakane *et al*. 2005). Infection induced strong host inflammatory reactions and antibody production against the parasite (Wang *et al*. 1997; Nakane *et al*. 2005). However, such immune responses were not effective enough to eliminate infection. *Neoheterobothrium hirame* first infects the gills of bastard halibut and moves to the buccal cavity wall for maturation. In infection experiments, halibut produced antibody against the parasite after the movement to the buccal cavity wall. Antibody production was further enhanced after death of the parasite induced a strong host reaction (Tsutsumi *et al*. 2003). How effectively the inflammatory response and subsequent antibody production by halibut induce immunity to re-infection remains to be studied.

Control methods

Control methods given below are not always applicable to every fish species and to all types of mariculture systems. Generally, application of a single method, if effective to some extent, will not be enough to control infection. Combinations of multiple methods are recommended.

Chemical treatment. Many chemical methods have been developed and used to control monogenean infections of maricultured fish. Although chemotherapy is applied on many occasions, it should be noted that development of resistance to selected chemical agents has been suggested in some freshwater monogeneans (Goven *et al*. 1980; Buchmann *et al*. 1992). Traditionally, freshwater bath treatment for up to 10 min has been practiced against *B. seriolae* infection of Japanese amberjack. This method is effective against monopisthocotyleans such as *B. seriolae* and *N. girellae* and can eradicate worms completely from fish. However, care should be taken that after the freshwater bath treatment, both greater amberjack and Japanese amberjack became more susceptible to re-infection with *N. girellae* (Ohno *et al*. 2009). Besides, where transport of fresh water to offshore cages is difficult, it is not practical for commercial scale treatment. It is to be noted that freshwater bathing is generally ineffective against polyopisthocotyleans.

NaCl-supplemented seawater bathing in a small tank was used to eradicate *N. hirame* from bastard halibut (Yoshinaga *et al*. 2000). More than 90 and 100% of immature worms were detached from the gills after 30 min and 60 min treatment, respectively, but it was not effective against adults on the buccal cavity wall. All fish were normal after the bathing, but this method can only be applied as a small-scale treatment.

Freshwater bathing and NaCl-supplemented seawater bathing are effective and inexpensive methods. However, there are some limitations in practice in the field as mentioned above. In this sense, chemicals which can be given orally as in-feed therapy or can be mixed freely with seawater as a bathing treatment have advantages over freshwater and NaCl-supplemented seawater bathing. In Japan, hydrogen peroxide bathing is approved against infections with *B. seriolae* of *Seriola* spp. at 660 ppm for 3 min, *N. girellae* and *H. okamotoi* of Japanese pufferfish at 660 ppm for 20 min and 1320 ppm for 20–30 min, respectively, and *B. tai* of red seabream at 660 ppm for 3 min. The use of this chemical is only allowed for the above combinations of parasites and fish under Japanese legislation. In Australia, hydrogen peroxide bathing is used to remove *Z. seriolae* from the gills of yellowtail amberjack at 300 ppm for 10 min (Mansell *et al*. 2005). Care must be taken because hydrogen peroxide increases its toxicity to the host at a high temperature (25 °C and higher).

Effective in-feed chemicals against marine monogeneans include a synthetic anthelminthic, praziquantel and benzimidazole-based compounds, mebendazole and febantel. Results of treatment trials with in-feed chemicals (Table 3) generally yielded varying rates of eradication from infected fish. Among them, praziquantel (PZQ) is the most widely used chemical, effective for both monopisthocotyleans and polyopisthocotyleans. However, care should be taken since medicated pellets can affect

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Table 3. In-feed administration of chemicals to reduce monogeneans from host

Chemicals	Parasites	Host fish	Dose	Efficacy (% reduction)	References
Praziquantel	*Benedenia seriolae*	*Seriola quinqueradiata*	150 mg kg^{-1} BW for 3 consecutive days	93·8–100%	Okabe (2000)
Praziquantel	*Benedenia seriolae*	*Seriola quinqueradiata*	150 mg kg^{-1} BW for 3 consecutive days	76·7–100%	Hirazawa *et al*. (2013)
Praziquantel	*Benedenia seriolae*	*Seriola dumerili*	150 mg kg^{-1} BW for 3 consecutive days	93·0–100%	Hirazawa *et al*. (2013)
Praziquantel	*Benedenia seriolae*	*Seriola lalandi*	50 or 75 mg kg^{-1} BW for 6 days	58·1–66·4%	Williams *et al*. (2007)
Praziquantel	*Neobenedenia girellae*	*Seriola quinqueradiata*	150 mg kg^{-1} BW for 3 consecutive days	35·9–76·5%	Hirazawa *et al*. (2013)
Praziquantel	*Neobenedenia girellae*	*Seriola dumerili*	150 mg kg^{-1} BW for 3 consecutive days	19·3–25·2%	Hirazawa *et al*. (2013)
Praziquantel	*Zeuxapta seriolae*	*Seriola dumerili*	50 or 75 mg kg^{-1} BW for 6 days	70·8–81·4%	Williams *et al*. (2007)
Praziquantel	*Microcotyle sebastis*	*Sebastes schlegeli*	200 mg kg^{-1} BW; single	about 58–100%	Kim *et al*. (1998); Kim and Cho (2000)
Praziquantel	*Sparicotyle chrysophrii*	*Sparus aurata*	158·1 mg kg^{-1} BW; single	31·0%	Sitjà-Bobadilla *et al*. (2006)
Mebendazole	*Microcotyle sebastis*	*Sebastes schlegeli*	50 or 100 mg kg^{-1} BW	52·3–71·9%	Kim and Choi (1998)
Bithionol	*Microcotyle sebastis*	*Sebastes schlegeli*	100 or 200 mg kg^{-1} BW	66·4–93·0%	Kim and Choi (1998)
Febantel	*Heterobothrium okamotoi*	*Takifugu rubripes*	25 mg kg^{-1} BW for 5 days	up to 80·9%	Kimura *et al*. (2009)

palatability of feed (Sitjà-Bobadilla *et al*. 2006; Williams *et al*. 2007). The efficacy of PZQ is significantly increased by administering cimetidine concurrently due to increased bioavailability of PZQ (Kim *et al*. 2001). Oral administration of mebendazole and bithionol were also used to remove *M. sebastis* from Korean rockfish (Kim and Choi, 1998; Kim *et al*. 1998). In Japan, febantel is an approved chemical against *H. okamotoi* infection of Japanese pufferfish (Kimura *et al*. 2009). Its administration is effective to remove both immature worms on the gills and adults on the branchial cavity wall. Oral intubation and bath administration of the above compounds are usually quite limited in practice. However, it is to be noted that bathing of 100 ppm PZQ for 4 min almost eradicated *M. sebastis* from Korean rockfish kept in a net cage (Kim and Cho, 2000).

Removal or inactivation of parasite eggs. Eggs of *B. seriolae* incubated at 30 °C did not hatch (Ernst *et al*. 2005). Hatching of the eggs of *N. girellae* kept in low salinities was suppressed (Umeda and Hirazawa, 2004). However, such temperature and salinity manipulations are usually not practical in net cage culture systems.

Monogeneans such as capsalids and most polyopisthocotyleans deposit eggs with a long filament or in the form of long strings, which can entangle with the net meshing. Hatched oncomiracidia from eggs on the culture net can easily encounter suitable hosts kept in the net. Once such monogeneans establish infection on cage-cultured fish, infection can rapidly spread among fish in the same cage and adjacent cages. To prevent heavy infections, frequent net change is commonly practiced to remove entangled eggs, where the host fish of the above monogeneans are cultured. Drying of nets is effective in killing eggs of *B. seriolae*, as no hatching of eggs was observed after desiccation of eggs for 3 min (Ernst *et al*. 2005).

Bastard halibut cultured in land-based, circular tanks are often infected with *N. hirame*. Unlike other typical polyopisthocotyleans, its eggs are provided with only short processes on both ends, which are unable to entangle with substrates within the tanks. The eggs can be washed out by overflowing culture water from the drain pipe in the centre of the tanks before hatch-out (Ogawa, unpublished observation).

Avoidance of infection source. In off-shore cage culture of yellowtail amberjack in Australia, tidal currents allow eggs/oncomiracidia of *B. seriolae* to reach amberjack kept 8 km away (Chambers and Ernst, 2005). This result indicates the importance of the cage location to effectively avoid infection. This also suggests that once amberjacks become infected in cages, it is extremely difficult to prevent infection from spreading. This is the main reason why

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B. seriolae infection is everywhere in amberjack farms of Japan.

Manipulation of culture methods, using knowledge of parasite biology, may provide, if not perfect, a solution for infection control. Juvenile greater amberjack placed in a shaded small experimental cage and exposed to *N. girellae* oncomiracidia had about 70% lower intensity of infection than those kept in a non-shaded cage (Shirakashi *et al*. 2013*a*). Juvenile greater amberjack were placed in a small cage and exposed to *N. girellae* oncomiracidia at depths of 0, 2 or 4 m. The intensity of infection was reduced by up to 80 and 95% in fish kept at depths of 2 and 4 m, respectively, compared with fish at 0 m (Shirakashi *et al*. 2013*b*). These results may reflect positive phototaxis by the oncomiracidium. Modifications of culture techniques such as shading and submergence of culture nets may represent effective control measures (Shirakashi *et al*. 2013*a*, *b*). Further studies will be needed to establish if these methods are as effective on a larger scale and if they are as effective to other monogenean species.

Selective breeding of fish strains with parasite resistance. Susceptibility of Japanese amberjack individuals to *B. seriolae* infection showed heritable variation, indicating that the host genes play a significant role in determining infection levels against the parasite (Nagakura *et al*. 2010). Through genome-wide and chromosome-wide linkage analyses using F_1 families of Japanese yellowtail based on a high-density linkage map with microsatellite and single nucleotide polymorphism markers, two major quantitative trait loci (QTL) regions were identified (Ozaki *et al*. 2013). The results will help resolve the mechanism of resistance to *B. seriolae* infection of Japanese yellowtail and could be used to breed resistant strains. Yellowtail culture in Japan is dependent on seed from wild stock. Selective breeding of resistant strains of artificially produced seeds may eventually replace wild-caught seed.

Other new approaches. An in-feed preventative agent can be a practical alternative to bathing treatment in capsalid infections. Barramundi, *Lates calcarifer*, were fed pellets supplemented with garlic extract for 30 days. When exposed to *Neobenedenia* sp. oncomiracidia, fish became infected up to 70% less than controls (Militz *et al*. 2013). Besides, no negative effect on palatability of the feed was recorded. Ohashi *et al*. (2007*a*) purified glycoproteins from skin mucus of Japanese pufferfish, which could induce attachment of *N. girellae* oncomiracidia. Ohashi *et al*. (2007*b*) successfully produced sterile *N. girellae* by introducing double-stranded RNA of *vasa*-related genes, essential for germ cell development. These results have not yet been applied on a larger scale for the control against this capsalid infection of maricultured fish.

DIGENEA

Digeneans (Platyhelminthes: Trematoda) infecting fish are hermaphrodites with the exceptions of some Didymozoidae, in which a male and female pair live together in a capsule. Life cycles of digeneans involve two or three hosts. Fish serve as intermediate hosts or final hosts. Adult worms are flat and leaf-shaped, have an oral sucker and a ventral one called an acetabulum, and infect various sites, mostly and typically alimentary tracts, of hosts. Blood flukes of fish, all belonging to Aporocotylidae, usually have no suckers; instead their lateral body is covered ventrally with rows of spines.

Most digeneans of fish are one to several mm in total length, with exceptionally large worms like some didymozoids of over 1 m (Bullard and Overstreet, 2008). The number of fish digeneans described is steadily increasing and will soon probably exceed the number of extant fish of about 28 000 species (Bullard and Overstreet, 2008). Important digeneans infecting maricultured fish are listed in Table 1.

Biology

As adult worms, fish digeneans are most common in the alimentary tracts of fish, and generally show negligible effects on their hosts, whereas aporocotylids representing blood flukes of fish infect the vascular system, sometimes causing mass mortalities of their hosts. Didymozoids form visible capsules in gills and visceral organs of marine fish (Fig. 4A), which are not harmful but may decrease or completely destroy the market value because of the unaesthetic appearance of infected fish.

Seasonality of digeneans of farmed marine fish is not always clear, as infection depends on the feeding of the fish on intermediate hosts harbouring metacercariae. Blood fluke infections are exceptions. Infection of farmed 0-year-old greater amberjack with *Paradeontacylix grandispinus* and *Paradeontacylix kampachi* (Aporocotylidae) was first detected in November, peaked in March and decreased toward July, judging by the number of accumulated eggs in the gills (Ogawa *et al*. 1993). A similar observation was made in *Cardicola aurata* (Aporocotylidae) infection of gilthead seabream, *S. aurata* cultured in Spain, in which the eggs in the gills were observed from November to May–June, with a peak in April (Holzer *et al*. 2008). These cases suggest that the cercarial invasion peaked in winter. Fish as second intermediate hosts of digeneans harbour metacercariae in various tissues and organs. Cercarial emergence from the first intermediate host and subsequent invasion into the fish host has seasonality (Paperna and Dzikowski, 2006), but seasonality in the metacercarial occurrence in fish becomes obscured, as they accumulate with fish age.

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Our knowledge of the generation time and longevity of digeneans infecting maricultured fish is limited. The culture industry of southern bluefin tuna, *Thunnus maccoyii*, in South Australia is based on the capture of 2–3-year-old wild fish and growing-out in sea cages for 2–8 months (Aiken *et al*. 2008). The lifespan of the blood fluke *Cardicola forsteri* in cultured southern bluefin tuna is estimated to be a minimum of 33 days to a maximum of 95 days (Aiken *et al*. 2009). Most culture seed of Pacific bluefin tuna are wild juveniles. Young Pacific bluefin tuna (300–930 g in body weight) caught in the Sea of Japan for culture seed were 100% infected with *Didymocystis wedli* (Didymozoidae) in the gills (Takebe *et al*. 2013). Regular monitoring of the infected fish after introduction into farms showed that the prevalence of infection kept decreasing and that the parasite capsules disappeared from fish within 5 months. This suggests that the didymozoid in the wild seeds will not live beyond the culture period. Further, no transmission of this digenean to other Pacific bluefin tuna kept in the farm occurred, suggesting the infection cycle was not established within the farm, and thus no special treatment was needed for control.

Blood fluke infections have been reported in many farmed fish species of Carangidae, Sparidae and Tetraodontidae. Despite big economic losses caused by these parasites, infections are not under control mainly because our knowledge of their life cycles is limited. The life cycles of only three species have been elucidated: *Aporocotyle simplex* (Aporocotylidae) infecting wild pleuronectid fishes (Køie, 1982; Køie and Petersen, 1988), *C. forsteri* infecting southern bluefin tuna and *Cardicola opisthorchis* infecting Pacific bluefin tuna (Sugihara *et al*. 2014). In all cases, the intermediate hosts are terebellid polychaetes, *Artacama proboscidea* and *Lanassa nordenskioeldi* for *A. simplex, Longicarpus modestus* for *C. forsteri* and *Terebella* sp. for *C. opisthorchis* (Køie, 1982; Køie and Petersen, 1988; Cribb *et al*. 2011; Sugihara *et al*. 2014). For *C. forsteri*, the intermediate host, though a single specimen was found infected, was collected from a sediment sample in the immediate vicinity of tuna cages (Cribb *et al*. 2011). The infected terebellid had hundreds of sporocysts in the coelom. The cercariae were small, with a mean body length of 71 μm and had a short tail. It was speculated that the cercariae are not active swimmers and are thus heavily dependent on currents for dispersal (Cribb *et al*. 2011).

Pathogenicity and associated host responses

Relatively few digenean species harm farmed fish (Bullard and Overstreet, 2008). Among the few harmful digeneans, blood flukes can cause mass mortalities of maricultured fish. For example, 50% to more than 80% of juvenile greater amberjack were lost in one month due to *P. grandispinus* and *P. kampachi* infection in Japan (Ogawa and Fukudome, 1994) and more than half of Japanese pufferfish were killed by an unidentified *Psettarium* (Aporocotylidae) (designated as *Psettarium* sp. TPC) within 3 months after puffers were introduced from China (Ogawa *et al*. 2007).

In Japan, cultured 0-year-old greater amberjack were heavily infected with *P. grandispinus* and *P. kampachi* in the heart and gill blood vessels. Their eggs accumulated in the gills, and the number sometimes exceeded 1 million eggs per fish (Ogawa *et al*. 1993). Dead fish were characterized by opened mouth and opercula, showing typical signs of asphyxiation (Fig. 4B) (Ogawa and Fukudome, 1994). Pathological changes were limited to the heart and gills with hyperplasia of gills, encapsulation of eggs in the gills and ventricle and papillate proliferation of the endothelium in the afferent branchial arteries (Fig. 3A) (Ogawa *et al*. 1989). Nodules were formed around the eggs, and extensive hyperplasia around them resulted in lamellar fusion, finally leading to hyperplastic clubbing of filaments (Fig. 3B). In spite of these host responses, most eggs in the gills developed normally to miracidia (Fig. 3B) and hatched out, whereas the eggs in the heart ventricle and most of the eggs at the base of the gill filaments were killed by the encapsulation and showed different stages of degeneration (Fig. 3C, D) (Ogawa *et al*. 1989). Crespo *et al*. (1992) reported mass mortality of juvenile greater amberjack cultured in Spain associated with unidentified blood flukes. Two species of *Paradeontacylix* have been described from *S. dumerili* in the Mediterranean, of which the causative blood fluke of the mass mortality could be *P. balearicus*, due to the presence of parasite eggs in the gill lamellae (Repullés-Albelda *et al*. 2008).

Similar pathological changes were observed in juvenile Pacific bluefin tuna infected with *Cardicola orientalis* and *C. opisthorchis* (Aporocotylidae) (Shirakashi *et al*. 2012*a*). Mixed infection with these two *Cardicola* species was common. The blood flukes could be identified by the morphology of the accumulated eggs in the gills; smaller, oval-shaped eggs, with a mean length of 44·0 μm, in the gill lamellae were produced by *C. orientalis* and larger, crescent-shaped eggs with a mean length of 52·1 μm that occurred primarily in the filamentary arteries, by *C. opisthorchis* (Shirakashi *et al*. 2012*a*). The number of eggs in the gills was highly variable among filaments. In a heavily infected 0-year-old fish, more than 9 million eggs were present.

Psettarium sp. TPC infected the visceral vascular system of farmed Japanese pufferfish, unlike the above cases of *Paradeontacylix* and *Cardicola*, which infect the heart and gill blood vessels of host fish (Ogawa *et al*. 2007). Eggs accumulated in visceral

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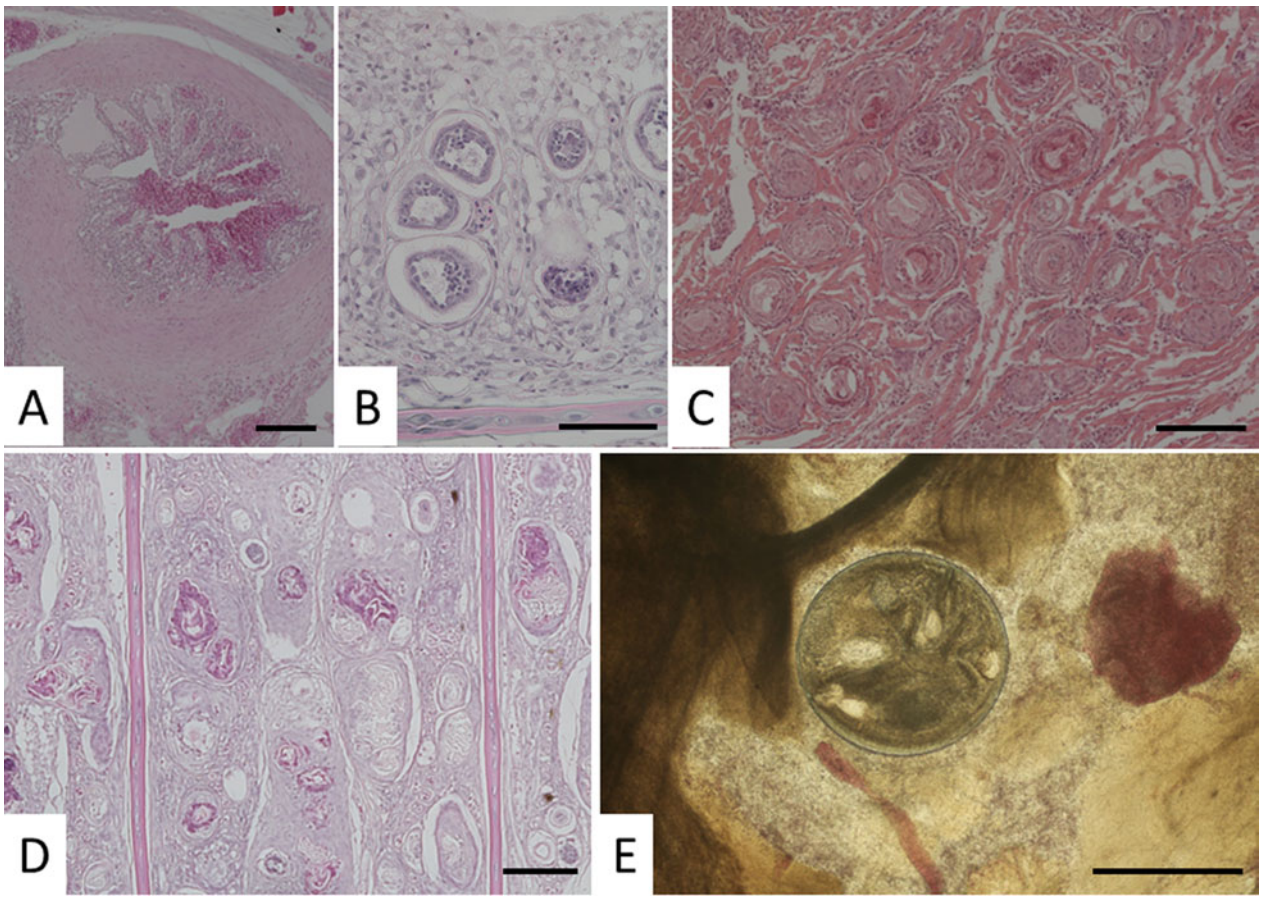

Fig. 3. Pathogenicity of digeneans – 1. (A) Cross section of an afferent branchial artery of greater amberjack, *Seriola dumerili*, heavily infected with *Paradeontacylix* spp. (scale: 0·1 mm); (B) *Paradeontacylix* eggs containing fully grown miracidia in the gill of greater amberjack (scale: 0·05 mm); (C) *Paradeontacylix* eggs encapsulated with the heart ventricle tissue of greater amberjack (scale: 0·1 mm); (D) Mass of encapsulated *Paradeontacylix* eggs at the basal part of gill filaments of greater amberjack (scale: 0·1 mm); (E) fresh brain tissue of red seabream, *Chrysophrys major* infected with *Galactosomum* sp. metacercaria (scale: 0·5 mm) (photo kindly provided by Mr Yukitaka Sugihara).

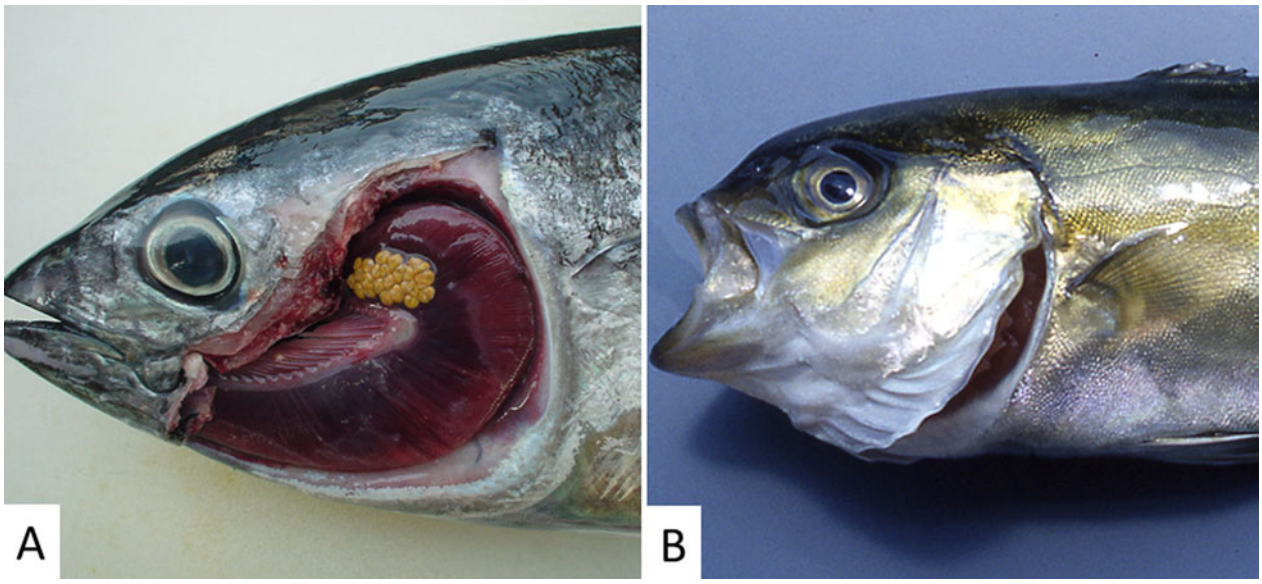

Fig. 4. Pathogenicity of digeneans – 2. (A) capsules of *Didymocystis wedli* on the gills of Pacific bluefin tuna, *Thunnus orientalis*; (B) suffocated juvenile greater amberjack, *Seiola dumerili* due to *Paradeontacylix* infection.

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organs such as the spleen, liver, testis, intestine and less frequently the gills. Egg masses in the visceral organs could sometimes be recognized as white spots in gross observations, suggestive of malfunction of these organs.

Very few metacercariae have been recorded as harmful parasites of maricultured fish, though intense metacercarial infections sometimes occur. Metacercariae of an acanthocolpid digenean *Stephanostomum tenue* (Acanthocolpidae) formed cysts in the bulbous arteriosus of maricultured rainbow trout, *Oncorhynchus mykiss* (as *Salmo gairdneri*). Degenerating larvae induced a severe inflammatory response and decreased respiration efficiency was responsible for mass mortalities of the host (McGladdery *et al*. 1990). Metacercariae of the galactosomid *Galactosomum* sp. (Galactosomidae) formed cysts in the brain of marine fish including Japanese amberjack, Japanese pufferfish, barred knifejaw, *Oplegnathus fasciatus* (Yasunaga *et al*. 1981) and red seabream (Fig. 3E), causing trematode whirling disease. Infected fish showed whirling swimming near the water surface. Diseased fish usually had one, rarely two, encysted metacercariae in the interbrain. Neurons around the metacercaria were degenerative or necrotic due to mechanical pressure of the cyst (Kimura and Endo, 1979). It is assumed that this abnormal swimming behaviour facilitates the final host, the black-tailed gull, *Larus crassirostris*, to find and catch the infected fish (Kamegai *et al*. 1982).

There are reports that fish infected with blood flukes acquire resistance or immunity against re-infection. One-year-old greater amberjack which had survived *Paradeontacylix* spp. infection in the previous year received milder infections than 0-year-old fish. The older fish may have acquired some immunity against blood fluke infection (Ogawa *et al*. 1989). Southern bluefin tuna cultured in Australia were able to control *C. forsteri* infection over a 6-month grow-out period (Aiken *et al*. 2006). Antibody response to *C. forsteri* was initiated after transfer of wild tunas to sea cages in April, and antibody titres reached a peak in December, when the infection drastically decreased from a peak in May (Aiken *et al*. 2008). Tuna farmed for 16 months had significantly lower prevalences and abundances of infection than those farmed for 5 months, showing the former group had acquired resistance against the blood fluke infection (Aiken *et al*. 2008).

Control methods

Chemotherapy. Preliminary experiments demonstrated that oral treatment of Pacific bluefin tuna with praziquantel (PZQ) was effective to control *C. opisthorchis* infection in the heart (Shirakashi *et al*. 2012*b*). The minimal effective dose for complete eradication was determined to be 7·5 mg kg^{-1} BW or higher for 3 consecutive days (Ishimaru *et al*. 2013). Repeated treatment may be required, as small numbers of adults re-appeared in experimental fish at 3 or 5 weeks post treatment (Shirakashi *et al*. 2012*b*). As noted in the control of monogenean infections with in-feed PZQ, medicated pellets can affect palatability of feed. PZQ is also effective to control *C. forsteri* infection of southern bluefin tuna though the minimum effective dose remains to be determined. A single oral intubation of PZQ at 75 mg kg^{-1} BW resulted in a significant reduction of the number of flukes in the hearts and eggs in the gills and myocardium (Hardy-Smith *et al*. 2012).

Avoidance of infection source. In the case of *S. tenue* metacercariae infection of rainbow trout (McGladdery *et al*. 1990), the first intermediate host *Nassarius obsoletus* and the final host, American eel *Anguilla rostrata*, were present in the vicinity of trout cages and removal of these natural hosts was impractical to avoid infection. Instead, placing net cages in water with over 7 m clearance from bottom successfully avoided cercarial invasion, reducing infection to a more or less negligible level (McGladdery *et al*. 1990).

Kirchhoff *et al*. (2011) monitored *C. forsteri* infection of southern bluefin tuna in two culture cages, one set near shore (about 30 km from shore; depth: 20 m) and the other offshore (about 46 km from shore; depth: 40 m). Six weeks after transfer of the cages to the two sites, offshore tuna had no *C. forsteri* infection, whereas a prevalence of 85% for the blood fluke was recorded in the near-shore tuna. The intermediate host, the terebellid polychaete, *L. modestus*, was collected from a bottom sample of 22 m in depth (Cribb *et al*. 2011). This polychaete is reported from the lower intertidal zone to a depth of 30 m (Hutchings and Glasby, 1988). It is possible then to reduce the blood fluke infection by physical separation from the intermediate host. From this, it may be deduced that cages should be set in deeper water as shown by Kirchhoff *et al*. (2011), though such offshore setting is inconvenient for maintenance of tuna. In contrast, *Terebella* sp. infected with sporocysts of *C. opisthorchis*, a blood fluke of Pacific bluefin tuna, was collected not only from the bottom sediments (46 m deep), but also from the ropes attached to the tuna cages (2 m deep) (Sugihara *et al*. 2014). In this case, relocating tuna cages away from the source of infection appears difficult.

CESTODA

Cestodes (Platyhelminthes: Cestoda) are hermaphrodites and endoparasites of vertebrates including fish. Typically the body is long and flat, consisting of the scolex, the attachment organ to host and the neck which generates segments that follow posteriorly.

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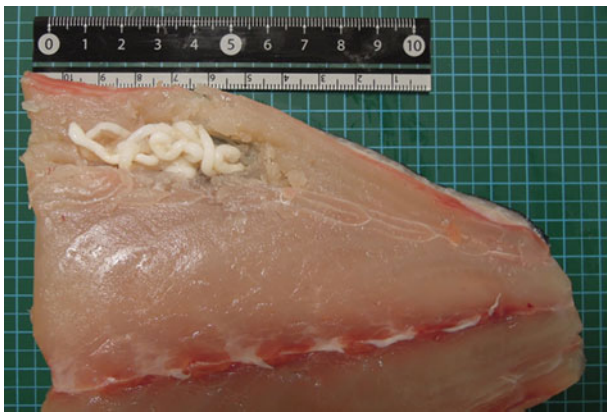

Fig. 5. A blastocyst of *Protogrillotia zerbiae* plerocercoid in the skeletal muscle of greater amberjack, *Seriola dumerili*.

They have two or three host life cycles. Fish serve as second intermediate hosts, infected through ingesting first intermediate hosts, or as final hosts, infected through ingesting second intermediate hosts. Second intermediate hosts harbour larval stages called plerocercoids or plerocerci in various tissues, whereas final hosts harbour adults in the alimentary tract.

Only a few cases of cestode infections are reported from maricultured fish (Table 1). Mortality of young chinook salmon *Oncorhynchus tshawytscha* cultured in net pens in Canada was caused by infections in the eye by plerocercoids of *Gilquinia squali* (Trypanorhyncha: Gilquiniidae) (Kent *et al*. 1991). The definitive host for *G. squali* is picked dogfish *Squalus acanthias* (as spiny dogfish *Squalus acanthus*). The life cycle is unknown, but chinook salmon presumably became infected by ingesting the infected first intermediate host, probably a crustacean. The lens of heavily infected fish was opaque, suggesting cataractous changes.

Some larval cestodes cause lowered market value due to infections in the edible part of the fish. A blastocyst of plerocercoid of *Trypanorhyncha* was found in the skeletal muscle of marketable-sized greater amberjack (Fig. 5), though the infection was very rare, with the prevalence of infection as low as one out of tens of thousands of fish processed (Ogawa *et al*. 2012). The cestode itself inside the blastocyst was not found, but molecular analysis of the small subunit ribosomal RNA gene revealed that the cestode to be *Protogrillotia zerbiae* (Lacistorhynchidae) (Tamaru, Klinger-Bowen, Ogawa, Iwaki, Kurashima and Itoh, unpublished data).

PROBLEMS ASSOCIATED WITH INTERNATIONAL TRADE OF MARINE FISH FOR AQUACULTURE

Some fish platyhelminthes have expanded their host ranges and geographical distributions through anthropological activities. A hitherto unknown *N. girellae* was first recorded from farmed marine fish of Japan in the 1990s (Ogawa *et al*. 1995*a*). The fact that juvenile greater amberjack imported from China as aquaculture seed was infected with this monogenean upon shipment to Japan and upon arrival in Japanese waters showed it is an introduced parasite. *Neobenedenia girellae* has been recorded from 15 species of farmed fish in Japan, posing a serious threat to Japanese aquaculture.

The diclidophorid *N. hirame* suddenly appeared in wild and farmed bastard halibut in Japan in the 1990s. First confirmation of infection was in 1993 on juvenile halibut collected in the Sea of Japan (Anshary *et al*. 2001). The parasite rapidly expanded its distribution to the Pacific side in 1997 (Ogawa, 2012), probably due to transfer of live juvenile and/or spawner halibut from the Sea of Japan side to the Pacific side for propagation and aquaculture purposes. Morphological and molecular evidence indicated that the original host for this monogenean is southern flounder *Paralichthys lethostigma*, naturally distributed on the Atlantic side of the USA (Yoshinaga *et al*. 2009). This suggests that *N. hirame* was introduced to the Far East with live southern flounder, though the route of introduction remains unspecified. This is an example of host switch by parasites induced by human activities.

Young Japanese pufferfish suffered mass mortalities caused by the blood fluke *Psettarium* sp. TPC, morphologically different from the indigenous *Psettarium* sp. TPJ (Ogawa *et al*. 2007). The host fish, imported from China to Japan, had already been infected, as the fish started to die within 3 days after arrival in Japan. Fortunately, no evidence was found that the parasite transmitted to wild and cultured domestic puffers.

PARASITE TRANSMISSION BETWEEN FARMED AND WILD FISH

Most parasites recovered from farmed fish are originally those of wild fish, and introduction of pathogens from the wild to farms is an important risk for sustainable aquaculture. Conversely, farmed fish may become sources of pathogens to wild fish, and release of pathogens from farms could negatively affect wild fish stocks. There are many reports and discussions on the parasite transmission between farmed and wild fish stocks, but problems lie in incongruence in the methods adopted for analysis of these phenomena (see Mladineo *et al*. 2009).

European seabass *D. labrax* and gilthead seabream farmed in the Mediterranean did not share platyhelminthes with farm-associated wild bogue *Boops boops* and Mediterranean horse mackerel *Trachurus mediterraneus* (Fernandez-Jover *et al*. 2010). Farming had no effect on the total parasite community between farm-associated and non-farm-associated wild bogue and mackerel, but may be detrimental for some parasite species, while these same

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conditions, such as diet modification, could enhance others (Fernandez-Jover *et al*. 2010).

Mariculture conditions can give host-specific parasites a chance to expand their host ranges. A heavy infection with the microcotylid *Polylabris tubicirrus*, known only from breams of the genus *Diplodus*, occurred among gilthead seabream kept in raceways using recirculating water (Silan *et al*. 1985). They speculated that the main cause of this unnatural infection was introduction of parasite eggs from the next raceway, where fish of the genus *Diplodus*, a natural host of this parasite, had been maintained. This is a case of host switch of a parasite, which occurred in a farm condition for the parasite to surmount the barrier of host specificity. The microcotylid *S. chrysophrii* is host specific to gilthead seabream. However, host switch of *S. chrysophrii* occurred from gilthead seabream to sharpsnout bream *Diplodus puntazzo* between net cages (Mladineo and Maršić-Lučić, 2007). Abundance of *S. chrysophrii* was greater in the new host than the original host. In contrast, comparing the mtDNA cytochrome oxidase I locus of *S. chrysophrii* collected from farmed gilthead seabream and those from wild bogue *Boops boops* (Mladineo *et al*. 2009) suggested that there was no transmission of this monogenean between farmed and wild fish in the Mediterranean.

Neoheterobothrium hirame infection of bastard halibut in Japan is another example of host switch by parasites, in which a parasite of wild fish in one country has transmitted to a different species of fish in a different country. With the introduction of this foreign parasite, both wild and farmed bastard halibut in Japan suffered heavy infections (Anshary *et al*. 2002), suggesting the monogenean transmits between wild and farmed halibut.

CONCLUSIONS

With a rapid expansion of the mariculture industry, many parasitic diseases have emerged among farmed marine fish. Some parasites have established their infection cycles within farms and it is not practically possible to eradicate them from farms. For monogeneans, egg filamentous appendages have a big advantage for their proliferation, as they entangle with net meshing and hatched larvae or oncomiracidia have much higher chances to encounter host fish in cages. A variety of control measures have been developed. A single method will not be enough and a combination of multiple methods is recommended for effective control of monogenean infections. Blood flukes are serious pathogens of farmed fish. Prevention of cercarial invasion into fish is hampered, as for most cases, their intermediate hosts have not yet been specified. In-feed administration of praziquantel is the only reliable control method. Chemotherapy is effective against monogenean and blood fluke infections, but can affect the health of farmed fish themselves. More effective and less harmful methods of parasite control within an ecosystem context that includes fish mariculture sites needs to be developed. Clearly, we have to expand our knowledge on the biology of important parasites of farmed fish to apply effective control.

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