Lat. Am. J. Aquat. Res., 45(4): 852-857, 2017
DOI: 10.3856/vol45-issue4-fulltext-24

Short Communication

Coinfection of *Caligus lalandei* and *Benedenia seriolae* on the yellowtail kingfish *Seriola lalandi* farmed in a net cage in northern Chile

Sandra Bravo[1], Carlos F. Hurtado[2] & María Teresa Silva[3]
[1]Universidad Austral de Chile, Puerto Montt, Chile
[2]Pontifícia Universidad Católica de Valparaíso, Valparaíso, Chile
[3]Universidad San Sebastián, Puerto Montt, Chile
Corresponding author: Sandra Bravo (sbravo@uach.cl)

ABSTRACT. The yellowtail kingfish *Seriola lalandi* is one of the most important fish candidates for the diversification of aquaculture in Chile. Fish farmed experimentally in one floating cage on a site located in Northern Chile, between November 2013 and December 2015, were first detected with the sea lice *Caligus lalandei* from April 2014. The highest prevalence (100%) was reached in July 2014 and the highest abundance in June 2014 (4.3 lice/fish). The monogenean *Benedenia seriolae* was also recorded in the same stock of fish from July 2014, reaching the highest abundance of 30.8 parasite/fish and a prevalence of 100% in January 2015. When the abundance of *B. seriolae* increased, *C. lalandei* abundance decreased until it disappeared in January 2015, which could be attributed to the increase of the seawater temperature in the period of study. This is the first report of *C. lalandei* infecting *S. lalandi* reared in net cage in Chile.

Keywords: *Seriola lalandi***,** *Caligus lalandei, Benedia seriolae***,** sea lice, monogenean ectoparasite, farmed kingfish.

The wild yellowtail kingfish *Seriola lalandi* has a migratory behaviour, with a world distribution covering Australia, New Zealand, Japan (Nugroho *et al*., 2001), South East China Sea (Randall & Lim, 2000), the Mediterranean Sea (Nakada, 2008) and the Pacific coast from Canada to Chile (Eschmeyer & Herald, 1999; Dyer & Westneat, 2010; Fernández *et al*., 2015). This wild species is annually recorded between November and April in northern Chile (20ºS-30ºS). (Sepúlveda & González, 2015a, 2015b). Commercial aquaculture production of yellowtail kingfish reared in net pen has been successfully established in Japan (Nakada, 2002), Australia (Hutson *et al*., 2007) and New Zealand (Moran *et al*., 2008), while in northern Chile, *S. lalandi* has been reared in the hatchery under controlled conditions since 2008.

Metazoan parasites have been shown to be an important threat for the yellowtail kingfish. The monogeneans *Zeuxapta seriolae* and *Benedenia seriolae* have been reported as the main parasitic problems of farmed kingfish (Ogawa & Yokoyama, 1998; Montero *et al*., 2004; Hutson *et al*., 2007). These flatworms can cause reduced appetite in heavy infections, slower growth and, in extreme cases, can cause death to the host if left untreated (Sharp *et al*., 2003; Whittington *et al*., 2011). *B. seriolae* is considered a major pathogen of farmed *S. lalandi* in South Australia and the major barrier to efficient production and industry growth (Chambers & Ernst, 2005; Lackenby *et al*., 2007), while in Japan it is estimated that *B. seriolae* is responsible for up to 22% of total production costs for the farmed Japanese yellowtail (*Seriola quinqueradiata)* (Ernst *et al*., 2002). In contrast, the copepod *Caligus lalandei* has been reported in New Zealand (Jones, 1998; Sharp *et al*., 2003) and in Japan infecting farmed yellowtail amberjack without serious problems (Ho *et al*., 2001). In Chile, the monogenean *Zeuxapta seriolae*, infecting the gill filament, and the copepod *C. lalandei* infecting the corporal surface have been reported on wild *S. lalandi* (Sepúlveda & González, 2015a, 2015b; Sepúlveda *et al*., 2016).

The aim of this study is to describe the coinfection of the sea lice *C. lalandei* and *B. seriolae* in a stock of *S. lalandi* reared for the first time in a sea cage in northern Chile (26º34'17"S, 70º41'29"W). In November 2013, 2,057 fish with a mean weight of 400 g, produced

Corresponding editor: Mauricio Laterça

Table 1. Prevalence, mean intensity and mean abundance of *Caligus lalandei* in the kingfish *Seriola lalandei*, per month and stage of development. n: number of sampled fish, SD: standard deviation, *P*: *P*-value, *No information was recorded.

Date		T°C	n	Prevalence (%)						Intensity						Abundance					
				Adult		Juvenile	Total	Z_c	*P*	Adult		Juvenile	Total	Z_c	*P*	Adult		Juvenile	Total	Z_c	*P*
				Male	Female					Male	Female		Mean ± SD			Male	Female		Mean ± SD		
2014	Jun	13.7	20	88.6	88.6	31.8	97.7			2.3	2.1	1.4	4.4 ± 2.3			2.0	1.8	0.4	4.3 ± 2.2		
	Jul	13.6	20	41.2	76.5	35.3	100.0	0.7	0.25 ns	1.6	1.8	1.0	2.4 ± 1.4	3.3	0.00 s	0.6	1.4	0.4	2.4 ± 1.4	3.2	0.00 s
	Aug*	13.7		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Sep	13.8	20	58.6	51.7	27.6	69.0	3.0	0.00 s	1.3	2.0	1.4	3.2 ± 3.1	1.2	0.12 ns	0.8	1.0	0.4	2.2 ± 2.1	0.4	0.34 ns
	Oct.	13.7	20	56.0	60.0	0.0	76.0	0.5	0.31 ns	1.9	1.7	0.0	2.7 ± 1.8	0.6	0.26 ns	1.0	1.0	0.0	2.0 ± 1.4	0.4	0.36 ns
	Nov	14.4	20	53.7	56.1	2.4	61.0	1.0	0.15 ns	1.3	1.3	0.0	2.4 ± 1.4	0.6	0.28 ns	0.7	0.7	0.0	1.5 ± 0.9	1.4	0.08 ns
	Dec	14.6	20	6.3	18.8	0.0	21.9	2.7	0.00 s	1.5	1.7	0.0	1.9 ± 7.4	0.3	0.38 ns	0.1	0.3	0.0	0.4 ± 1.6	2.7	0.00 s
2015	Jan	15.6	20	0.0	0.0	0.0	0.0	2.4	0.01 s	0.0	0.0	0.0	0.0	1.1	0.13 ns	0.0	0.0	0.0	0.0	1.1	0.13 ns
	Feb	15.3	20	0.0	0.0	0.0	0.0	0	0	0.0	0.0	0.0	0.0			0.0	0.0	0.0	0.0		

in captivity under controlled conditions in a hatchery with a recirculating system, were transferred to a circular submersible cage made with copper alloy mesh (20 m diameter and 10 m depth). When fish reached a mean weight of 1,250 g in April 2014, the ectoparasite *C. lalandei* was recorded infesting farmed fish for the first time in a routine sample weight. After that finding, 20 fish were monitored monthly to evaluate the abundance of parasites in the fish population (Table 1). Prevalence was calculated as the percentage of infected fish in the sample, mean abundance as the mean number of parasites per fish examined, and the mean intensity as the mean number of parasites per infected fish (Rózsa *et al*., 2000).

Fish were collected with a hand net, and then anaesthetised with benzocaine (10% in ethanol, 1 mL^{-1}) prior to individual examination by eye. Parasites were counted and classified according to gender and stage of development (female, male and juvenile), and fish were returned carefully to the same cage after sampling. Parasites were stored in 95% ethanol for later examination, and subsequently identified according to morphological features.

The identity of *C. lalandei* (Fig. 1) was confirmed by comparison with the paratypes of *C. lalandei* in the collections of The Natural History Museum, London, while the identity of *B. seriolae* (Fig. 2) was confirmed by consulted bibliography (Baeza & Castro, 1975; Kearn *et al*., 1992; Kinami *et al*., 2005). The Z test for significant differences of proportion was used to assess differences in prevalence between months, and the *t*-test to assess differences in abundance and intensity between months.

In the period of study, the temperature ranged between 13.6 (July 2014) and 15.3ºC (February 2015), and salinity ranged between 34.3 and 34.7. The highest prevalence of *C. lalandei* was recorded in July 2014 (100%), when *B. seriolae* was recorded infecting the same stock of fish. Since that date, the prevalence of *C. lalandei* decreased to 0% in January 2015, while the prevalence of *B. seriolae* increased from 5.9% in July to 100% in January 2015 (Fig. 3).

No data were collected in August 2014 because of winter storm conditions. The highest abundance and intensity of infection of *C. lalandi* was recorded in June 2014 (Figs. 4-5) when the seawater temperature reached 13.7ºC, decreasing through the months to reach an abundance of 1.1 lice/fish and an intensity of infection of 1.9 lice/fish in December 2014 when the mean temperature was 14.6ºC (Table 1). No *Caligus* were recorded in January and February, while the monogenean *B. seriolae* was recorded from July 2014 with an abundance of 0.1 parasite/fish (Table 2). In contrast to *C. lalandei*, the abundance of *B. seriolae*

Figure 1. *Seriola lalandi* infected with gravid female of *Caligus lalandei* (Arrow).

Figure 2. *Seriola lalandi* infected with *Benedenia seriolae* (Arrows).

increased through the following months to reach 30.8 parasites/fish in January 2015, when temperature reached 15.6ºC (Fig. 4). There was no mortality or damage to the skin associated with the infestation of *C. lalandei* during the period of study, nor to *B. seriolae*. Therefore no treatment was required to control these parasites on fish kept at a density lower than 1 kg m^{-3}. This farm experiment was completed when fish reached an average weight of 2.55 kg in February 2015.

Although *S. lalandi* has been reared in captivity in northern Chile since 2008 no parasites were previously reported in farmed fish as this species was only reared under controlled conditions in a hatchery with a recirculating system. The infestation recorded on farmed *S. lalandi* in sea cage by *C. lalandei* and *B. seriolae*, can be explained by the presence of the wild kingfish *S. lalandi* in the area where the cage was settled (*pers. observ*.). In fact *B. seriolae* was reported infecting the wild kingfish *S. lalandi* in northern Chile

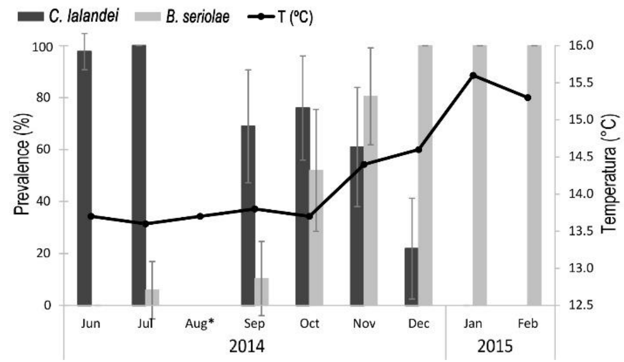

Figure 3. Monthly prevalence and confidence interval 95% of *Caligus lalandei* and *Benedenia seriolae* in the kingfish *Seriola lalandei* in the period of study, in relation to the seawater temperature. No information was recorded in August 2014.

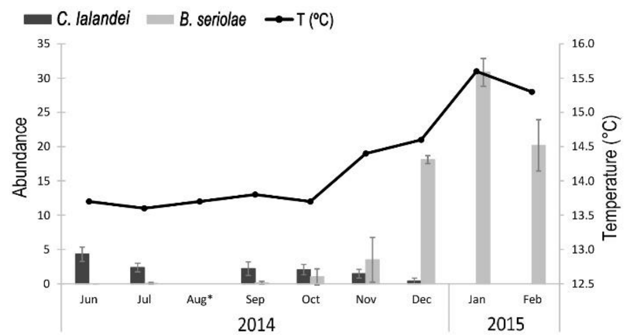

Figure 4. Mean abundance and confidence interval 95% of *Caligus lalandei* and *Benedenia seriolae* infecting the kingfish *Seriola lalandei* in the period of study, in relation to the seawater temperature. No information was recorded in August 2014.

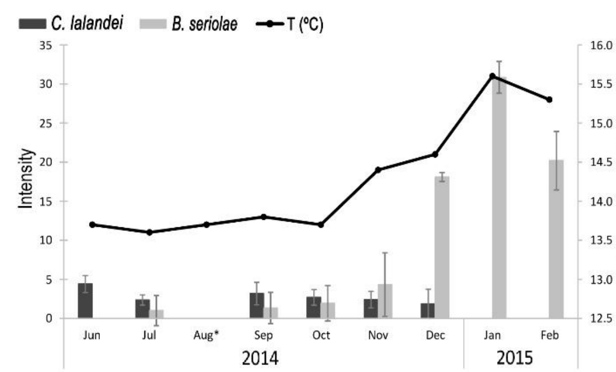

Figure 5. Mean intensity and confidence interval 95% of *Caligus lalandei* and *Benedenia seriolae* infecting the kingfish *Seriola lalandei* in the period of study, in relation to the seawater temperature. No information was recorded in August 2014.

for the first time in 1975 (Baeza & Castro, 1975) and *C. lalandei* in 1980 (Baeza & Castro, 1980). A similar situation was reported in Australia, where fingerlings

Table 2. Prevalence, mean intensity and mean abundance of *Benedenia seriolae* in the kingfish *Seriola lalandei*, per month and stage of development. n: number of sampled fish, SD: standard deviation. *No information was recorded.

Date		T°C	n	Prevalence (%) Adult	Juvenile	Total	Z_c	P	Intensity Adult	Juvenile	Mean ± SD	Z_c	P	Abundance Adult	Juvenile	Mean ± SD	Z_c	P
2014	Jun	13.7	20	0.0	0.0	0.0	-	-	0.0	0.0	0.0	-	-	0.0	0.0	0.0	-	-
	Jul	13.6	20	5.9	0.0	5.9	1.1	0.13 ns	1.0	0.0	1.0 ± 4.1	18.4	0.00 s	0.1	0.0	0.1 ± 0.2	1.8	0.03 s
	Aug*	13.7		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Sep	13.8	20	10.3	0.0	10.3	0.5	0.30 ns	1.3	0.0	1.3 ± 4.3	2.7	0.00 s	0.1	0.0	0.1 ± 0.4	0.0	0.50 ns
	Oct.	13.7	20	52.0	0.0	52.0	3.2	0.00 s	1.9	0.0	1.9 ± 4.8	1.0	0.15 ns	1.0	0.0	1.0 ± 2.5	1.6	0.06 ns
	Nov	14.4	20	73.2	34.1	80.5	2.0	0.02 s	3.1	3.6	4.3 ± 8.6	1.5	0.07 ns	2.3	1.2	3.5 ± 6.9	1.5	0.07 ns
	Dec	14.6	20	100.0	93.8	100.0	2.2	0.01 s	11.2	7.4	18.1 ± 1.2	8.8	0.00 s	11.2	6.9	18.1 ± 1.2	9.3	0.00 s
2015	Jan	15.6	20	100.0	100.0	100.0			5.2	25.6	30.8 ± 4.3	12.7	0.00 s	5.2	25.6	30.8 ± 4.3	12.7	0.00 s
	Feb	15.3	20	100.0	95.2	100.0			15.9	4.6	20.2 ± 7.9			15.9	4.3	20.2 ± 7.9		

reared in land-based hatcheries were parasite-free but, when they were transferred to sea-cages for grow out, fluke populations proliferated due to their direct life cycle and the reservoir of infections on wild *S. lalandi* (Whittington *et al*., 2011).

B. seriolae is a cosmopolitan species reported as the most pernicious parasite of farmed kingfish around the world (Ogawa & Yokoyama, 1998; Whittington *et al*., 2001; Tubbs *et al*., 2005). Therefore medicinal treatments such as freshwater baths and hydrogen peroxide are used for its control (Chambers & Ernst, 2005). Although no mortality was associated with this parasite in this study, a reduction in growth was recorded since December 2015, which could be associated in part to the *Benedenia* infestation, such as has been reported by other authors (Whittington, 2005; Whittington & Chisholm, 2008).

C. lalandei is also considered a cosmopolitan species reported for the first time from wild *S. lalandi* caught in Kalk Bay, South Africa (Barnard, 1948). Subsequently, it was reported from the same wild fish species in Chile (Baeza & Castro, 1980) and New Zealand (Jones, 1998; Sharp *et al*., 2003). *C. lalandei* infecting farmed yellowtail was reported for the first time in Japan without serious problem associated with disease, although Ho *et al.* (2001) suggested it may cause a serious problem in the event of an outbreak because of its large size in comparison with other *Caligus* species.

In this study, fish did not show physical damage that would require any medicinal intervention to control both parasite species, perhaps because fish were reared at a density that did not exceed 1 kg m^{-3} in the experimental sea-cage. The most relevant issue was that the higher prevalence and abundance of *C. lalandei* was recorded in the winter period, declining until it was absent in the summer months (Table 1), when the prevalence and abundance of *B. seriolae* increased (Table 2), which seems to be associated with the seawater temperature (Figs. 3-4). In fact, Ernst *et al*. (2005) demonstrated that temperature strongly influenced the embryonic period in *B. seriolae*. At high temperature the eggs hatch earlier than at low temperature, which can explain the increased abundance in the summer period. In contrast, there is no antecedent that could explain the declining abundance of *C. lalandei* in the same period.

This situation will be examined in further studies to understand the epidemiological behaviour of both parasites, and the factors affecting the prevalence and abundance on *S. lalandi*. This will enable procedures and disease management strategies to be developed, in order to minimize future losses during the rearing period in sea cages.

ACKNOWLEDGMENTS

We thank to Dr. Geoffrey Boxshall from The Natural History Museum, London who confirmed the identity of *Caligus lalandei*, to Ecosea Farming S.A., which is carried out the project with *Seriola lalandi* and provided us with the lice and information from the farm, and to Dr. James Treasurer for his kind help with the revision of the manuscript.

REFERENCES

Baeza, H. & R. Castro. 1975. *Benedenia melleni* (MacCallum, 1927) ectoparásito en peces de Antofagasta (Platyhelminthes, Monogenea). Apuntes Oceanol., 7: 14-22.

Baeza, H. & R. Castro. 1980. Tres especies de *Caligidae* nuevas para la fauna chilena (Copepoda, Siphonostomatoida). Not. Mens. Mus. Nac. Hist. Nat., 25: 3-7.

Barnard, K.H. 1948. New records and descriptions of new species of parasitic Copepoda of South Africa. Ann. Mag. Nat. Hist. Ser., 12(1): 242-254.

Chambers, C. & I. Ernst. 2005. Dispersal of the skin fluke *Benedenia seriolae* (Monogenea: Capsalidae) by tidal currents and implications for sea-cage farming of *Seriola* spp. Aquaculture, 250: 60-69.

Dyer, B.S. & M.W. Westneat. 2010. Taxonomy and biogeography of the coastal fishes of Juan Fernández Archipelago and Desventuradas Islands, Chile. Rev. Biol. Mar. Oceanogr., 45: 589-617.

Ernst, I., I. Whittington, S. Corneillie & C. Talbot. 2002. Monogenean parasites in sea-cage aquaculture. Austasia Aquacult., 16: 46-48.

Ernst, I., I. Whittington, S. Corneillie & C. Talbot. 2005. Effects of temperature, salinity, desiccation and chemical treatments on egg embryonation and hatching success of *Benedenia seriolae* (Monogenea: Capsalidae), a parasite of farmed *Seriola* spp. J. Fish Dis., 28: 157-164.

Eschmeyer, W.N. & E.S. Herald. 1999. A field guide to Pacific coast fishes: North America. Houghton Mifflin Harcourt, New York, 352 pp.

Fernández, G., D. Cichero, A. Patel & V. Martínez. 2015. Genetic structure of Chilean populations of *Seriola lalandi* for the diversification of the national aquaculture in the north of Chile. Lat. Am. J. Aquat. Res., 43(2): 374-379.

Ho, J.S., K. Nagasawa, I.H. Kim & K. Ogawa. 2001. Occurrence of *Caligus lalandei* Barnard, 1948 (Copepoda, Siphonostomatoida) on amberjacks (*Seriola* spp.) in the western north Pacific. Zool. Sci., 18: 423-431.

Hutson, K.S., I. Ernst & I.D. Whittington. 2007. Risk assessment for metazoan parasites of yellowtail kingfish *Seriola lalandi* (Perciformes: Carangidae) in south Australian sea-cage aquaculture. Aquaculture, 271: 85-99.

Jones, B. 1998. New Zealand parasitic Copepoda; genus *Caligus* Muller, 1785 (Siphonostomatoida: Caligidae). New Zeal. J. Zool., 15: 397-413.

Kearn, G.C., K. Ogawa & Y. Maeno. 1992. Egg production, the oncomiracidium and larval development of *Benedenia seriolae*, a skin parasite of the yellowtail, *Seriola quinqueradiata*, in Japan. Publ. Seto, Mar. Biol. Lab., 35(6): 351-362.

Kinami, R., J. Miyamoto, T. Yoshinaga, K. Ogawa & Y. Nagakura. 2005. A practical method to distinguish between *Neobenedenia girellae* and *Benedenia seriolae*. Fish Pathol., 40: 63-66.

Lackenby, J.A., C.B. Chambers, I. Ernst & I.D. Whittington. 2007. Effect of water temperature on reproductive development of *Benedenia seriolae* (Monogenea: Capsalidae) from *Seriola lalandi* in Australia. Dis. Aquat. Organ., 74: 235-242.

Montero, F., E. Crespo, S. Padrós, F. De la Gándara, F.A. García & J.A. Raga. 2004. Effects of the gill parasite *Zeuxapta seriolae* (Monogenea: Heteraxinidae) on the amberjack *Seriola dumerili* Risso (Teleostei: Carangidae). Aquaculture, 232: 153-163.

Moran, D., R.M. Wells & S.J. Pether. 2008. Low stress response exhibited by juvenile yellowtail kingfish (*Seriola lalandi* Valenciennes) exposed to hypercapnic conditions associated with transportation. Aquacult. Res., 39: 1399-1407.

Nakada, M. 2002. Yellowtail culture development and solutions for the future. Rev. Fish Sci., 10: 559-575.

Nakada, M. 2008. Capture-based aquaculture of yellowtail. In: A. Lovatelli & P.F. Holthus (eds.). Capture-based aquaculture. Global overview. FAO Fish. Tech. Pap., 508: 199-215.

Nugroho, E., D.J. Ferrell, P. Smith & N. Taniguchi. 2001. Genetic divergence of kingfish from Japan, Australia and New Zealand inferred by microsatellite DNA and mitochondrial DNA control region markers. Fish. Sci., 67: 843-850.

Ogawa, K. & H. Yokoyama. 1998. Parasitic diseases of cultured marine fish in Japan. Fish Pathol., 33(4): 303-309.

Randall, J.E. & K.K.P. Lim. 2000. A checklist of the fishes of the South East China Sea. Raffles Bull. Zool., S8: 569-667.

Rózsa, L., J. Reiczigel & G. Majoros. 2000. Quantifying parasites in samples of hosts. J. Parasitol., 86(2): 228-232.

Sepulveda, F.A. & M.T. González. 2015a. Patterns of genetic variation and life history traits of *Zeuxapta* *seriolae* infesting *Seriola lalandi* across the coastal and oceanic areas in the south eastern Pacific Ocean: potential implications for aquaculture. Parasites Vectors, 8: 282 pp. doi 10.1186/s13071-015-0892-4.

Sepulveda, F.A. & M.T. González. 2015b. Molecular and morphological analyses reveal that the pathogen *Benedenia seriolae* (Monogenea: Capsalidae) is a complex species: implications for yellowtail *Seriola* spp. Aquaculture, 418-419(2014): 94-100.

Sepulveda, F.A., J.F. Torres, C.D. Infante & M.T. González. 2016. Potential role of ectoparasites (*Zeuxapta seriolae* and *Caligus lalandei*) in the transmission of pathogenic bacteria in yellowtail kingfish *Seriola lalandi*, inferred from cultivable microbiota and molecular analyses. J. Fish Dis. doi:10.1111/jfd.12582.

Sharp, N., J. Poortenaar, C.W. Diggles, B.K. Diggles & T.J. Willis. 2003. Metazoan parasites of yellowtail kingfish, *Seriola lalandi lalandi*, in New Zealand: prevalence, intensity, and site preference. New Zeal. J. Mar. Freshw. Res., 37: 273-282.

Tubbs, L., C. Poortenaar, M. Sewell & B. Diggles. 2005. Effects of temperature on fecundity *in vitro*, egg hatching and reproductive development of *Benedenia seriolae* and *Zeuxapta seriolae* (Monogenea) parasitic on yellowtail kingfish *Seriolae lalandi*. Int. J. Parasitol., 35: 315-327.

Received: 13 January 2017; Accepted: 20 April 2017

Whittington, I.D. 2005. Monogenea Monopisthocotylea (ectoparasitic flukes). In: K. Rohde (ed.). Marine parasitology. CSIRO Publishing, Melbourne, pp. 63-72.

Whittington, I.D. & L.A. Chisholm. 2008. Diseases caused by Monogenea. In: J.C. Segner, H. Wahlii & B.G. Kapoor (eds.). Fish diseases. Eiras Science Publishers, New Hampshire, pp. 683-816.

Whittington, I.D., A.P. Shinn, J.E. Bron & M.R. Deveney. 2011. Innovative solutions for aquaculture: assessment of *in situ* monitoring techniques and life history parameters for monogenean skin and gill parasites. Final Report to FRDC (Project Nº2003/221). University of Adelaide, Adelaide, South Australia, 41 pp.

Whittington, I.D., S. Corneillie, C. Talbot, J. Morgan & R. Adlard. 2001. Infections of *Seriola quinqueradiata* Temminck & Schlegel and *S. dumerili* (Risso) in Japan by *Benedenia seriolae* (Monogenea) confirmed by morphology and 28S ribosomal DNA analysis. J. Fish Dis., 24: 421-425.