47

Techniques for Addressing Parasites in Saltwater Aquariums

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Parasitic infections of fish are common and are often secondary opportunistic infections; therefore it is recommended to establish a thorough quarantine protocol (Box 47.1) in efforts to reduce the risks of introducing parasitic infections to an established aquarium system. Occasionally, breakthrough parasitic infestations still occur, despite an intensive quarantine protocol, due to parasite life cycle, anatomic location of parasite on affected animal, or treatment failure. Implementing a thorough routine preventative medicine protocol (Box 47.2) may facilitate diagnosis and guide treatment and/or management decisions for parasitic infections of established fish collections (see also Chapter 49).

Diagnostics

Diagnostics should first consist of evaluation of the environment, water quality, and visual observation of the fish. Clinical signs of parasitic infections may be nonspecific and may include the following: abnormal/erratic swimming, flashing (i.e., rubbing body across surfaces), clamped fins (i.e., holding fins close to body), increased respiratory rate and/or piping (i.e., gulping at surface), color changes to skin and/or gills, excessive mucus on skin and/ or gills, raised nodules, ulcerative and/or erosive lesions on the skin, loss of scales and/or fins, exophthalmia and/ or cloudy eyes, coelomic distension, emaciation, or gross visual observation of large external parasites. The majority of fish diseases can be diagnosed by evaluation of water quality and wet mount examination of tissues with light microscopy.6 Therefore skin scrapes, fin clips, and gill biopsy wet mount examination with light microscopy should be considered the minimum diagnostic database for most species of fish when presented for a physical examination. To avoid potential parasitic detachment that could decrease the sensitivity of your diagnostic test, when possible, it is best to acquire these samples prior to sedation, while ensuring human and animal safety are still prioritized.6 When there is high morbidity and mortality in a collection of fish and ante mortem diagnostics have not revealed a

definitive diagnosis, humane euthanasia and necropsy on a subset of fish, including wet mount examination of internal organs and histopathology, may be considered a reasonable diagnostic tool. Other diagnostics that may be pursued to evaluate for the presence of parasites in some saltwater fish species include fecal examination, freshwater dip, endoscopy, and coelomic saline flush. Identification of the parasite to species or genus is not always required to determine an initial therapeutic course of action.⁶

Treatment

The sensitivity of skin, fin, and gill wet mount diagnostic examinations may be low in subclinical or minimally affected fish because the acquired sample represents only a small portion of the actual tissue; therefore a negative test result does not ensure that the population of fish examined are truly parasite free. For this reason, prophylactic treatments may be used as part of a quarantine protocol to prevent the introduction of parasites into an established system. 3.4

Numerous factors must be taken into consideration when deciding on a treatment plan besides the target parasite. Environmental factors (e.g., temperature, salinity, dissolved oxygen, alkalinity) may influence treatment and monitoring. It is important to know, for example, that for each 5 mg/L of formalin added to a system, it depletes 1 mg/L of oxygen.1 Thus, increasing aeration and closely monitoring dissolved oxygen are essential when treating with formalin, especially at high temperatures. The decision to treat individual fish or an entire aquarium system/population often guides the veterinarian's medication selection and desired route of administration. When oral medications are chosen, the animal's current appetite and the food preference of the species typically guides the formulation selected (e.g., bioencapsulation in brine shrimp, medicated flakes or gel, gruel). The life cycle of the target parasite influences treatment frequency and duration. Oviparous parasitic species usually require multiple repeated treatments and a longer duration of treatment when compared with viviparous parasitic species. Furthermore, it is important to know

• BOX 47.1

Quarantine Considerations for Fish Maintained in Public Display Aquaria

All new incoming fish species should be placed in quarantine for a minimum of 30 days (45–60 days usually recommended for cold water species).

Entrance and Exit Examinations

Entrance and exit examinations on a subset of the population (~2%-5%) should occur within 1 week of quarantine arrival and 1 week prior to quarantine departure. The following criteria should be met when possible during both of those examinations:

- Visual inspection
- Body weight
- Skin scrape (+/- fin clip) wet mount examination
- · Gill clip wet mount examination when feasible
- Blood sampling for hematology and plasma biochemistry, especially in moray eel and elasmobranch species (not recommended in fish <8 cm total length)
- Thorough necropsy of all deceased fish when possible including postmortem wet mount samples of skin scrapes, fin clips, all biopsies, and all internal organs

Treatments

Treatment protocols should be designated by veterinary staff. This may include prophylactic treatment of quarantined fish to prevent parasitic introduction to established systems (e.g., praziquantel, formalin, fenbendazole, copper) and/or specific treatment based on diagnostic examinations.

Medical Records

Written or computerized records should be maintained for each system documenting the following criteria daily to monitor trends during the quarantine period:

- Water quality parameters
- Number of mortalities
- Treatments administered (e.g., dose, duration/frequency, and route)
- · Estimated percentage of food intake for the population

Biosecurity

- Fish should ideally be quarantined in an isolated facility located away from collection animals
- Teleost fish, elasmobranchs, and invertebrate species should be quarantined in separate systems by taxa when possible
- There should be designated staff members assigned to work only in the quarantine facility. Alternatively, if that is not possible, collection fish should be handled first prior to employees entering the quarantine facility for the day
- Foot baths should be maintained at all quarantine entryways and exits, and if possible, between systems
- Designated nets and equipment should be assigned to each system. Alternatively, if that is not possible, gloves, hand washing stations, and net disinfection stations should be readily available and easy to access

This information was compiled and summarized from previous published literature on fish quarantine protocols; therefore the reader is encouraged to refer to these references for complete details.^{3,4}

• BOX 47.2

Routine Preventative Medicine Considerations for Fish Maintained in Public Display Aquaria

Elasmobranchs

Routine Health Evaluations

At minimum all elasmobranchs should be visually examined by a staff veterinarian annually. When feasible, handling an elasmobranch for a brief physical examination once a year is advised and should be discussed among staff veterinarians and aquarium leadership. The decision to handle should be based on species, human and animal safety, enclosure and ability to catch, available equipment, and available space. If handled for a physical examination, the following sampling criteria should be met when possible:

- Appropriate restraint for select species to ensure both animal and human safety (e.g., tonic clonic immobilization, oxygen narcosis, behavioral or manual restraint in a net or sling, or chemical)
- · Body weight and morphometric measurements
- Blood sampling for hematology, plasma chemistries, and point-of-care analyzer (e.g., blood gasses and lactate)
- Skin scrape and gill biopsy wet mount examination when possible
- Ultrasound examination, especially females
- Coelomic wash for species considered susceptible to Eimeria southwelli (e.g., cownose rays)
- Radiographs for select species (e.g., sand tiger sharks with suspected spinal deformity)

Teleost Fish

Routine Health Evaluations

At minimum all teleost fish should be visually examined by a staff veterinarian annually. If a fish is handled for any cause, the following minimal physical examination criteria should be performed when feasible:

- Appropriate restraint for select species to ensure both animal and human safety (e.g., manual restraint in a net/sling or chemical)
- Weight and appropriate morphometric measurements
- Skin scrape (+/- fin clip) wet mount examination
- · Gill clip wet mount examination when feasible
- Blood sampling for hematology and plasma biochemistry when possible (not recommended in fish <8 cm total length)

the consequences the selected treatment may have on the filtration, microbe community, and plants in the exhibit and species-specific sensitivities to the medication prior to treating. If this knowledge is not known, it is advised to consult with experienced colleagues, treat a smaller number of fish, or use surrogate species and monitor them closely prior to treating an entire system or population to avoid unexpected adverse results.¹

Treatment for parasitic infections may consist of environmental manipulation (e.g., temperature or salinity changes), the addition of biological control (e.g., cleaner fish), manual removal of parasites, and chemical medications. It is usually recommended to treat the entire aquarium environment, when possible and when applicable, for the best chance of successful therapy. However, once parasites are introduced to an established system, treatment may become problematic, especially when involving large mixed-species aquariums

with complex life support systems. When that does occur, efforts are usually focused on parasitic management by maintaining animal health and appropriate temperature, water quality, filtration, substrate, and stocking density for the housed aquarium species to prevent parasitic flare-ups and subsequent morbidity and mortality. Indefinite hyposalinity (Table 47.1) has been a valuable tool for the successful management of Neobenedinia sp. in large mixed-species exhibits containing elasmobranchs and teleost fish. There has also been evidence to suggest that short-term use of hyposalinity, along with temperature increases, may aid in resolution of Cryptocaryon irritans infections in large tropical marine exhibits as well (K. Heym, personal communication, March 16, 2017). In cases of parasitic flare-ups in which a specific species is markedly compromised and the entire system cannot be treated, it may become necessary to remove those fish from the environment and treat individually. Manual removal of parasites, short-term dips and baths, medications administered via gastric lavage, and parenteral treatment are often reserved for treating individual fish or anorectic fish. Because parasitic infections are often secondary and opportunistic, it is important to complete a thorough physical examination on heavily parasitized fish to determine if other medications (e.g., antimicrobials) and supportive care are needed in addition to antiparasitic agents.

Challenges and Novel Treatment Considerations

Degradation of Praziquantel and Formalin in a Recirculating System

Research has discovered significant degradation of both praziquantel and formalin treatments in saltwater aquariums when using standard published doses and frequencies. Failure to maintain therapeutic concentrations in a system could lead to decreased efficacy, recurrence of pathogen, and possibly the development of resistance. The microbial population within the aquarium system is thought to be the primary contributing factor for the degradation of these treatments. Degradation rates appear to increase with subsequent dosing of these medications, possibly related to increased bacterial activity or bacterial growth that may use these medications as an energy source after the first exposure.

Investigations have demonstrated that an initial dose of praziquantel at 2 mg/L and formalin at 25 mg/L degraded to less than detectable limits in naïve recirculating saltwater systems in approximately 9 days and 14 hours, respectively. The rate of removal for each of those medications continued to increase with subsequent treatments at those same doses. So Similar degradation has been observed in clinical settings, with praziquantel reaching nondetectable levels in as little as 8 hours in a freshwater aquarium (M. Hyatt, personal communication, February 27, 2017) and in as little as 4–6 hours in a saltwater aquarium (S. Boylan, personal communication, February 27, 2017). These investigations emphasize the significance of monitoring

drug concentrations throughout treatment to determine appropriate dosage frequencies and to ensure therapeutic levels are maintained. Removing fish from an experienced system into a naïve system or enclosure for each treatment may help to minimize rapid degradation.

Capsalid Management in a Large Mixed-Species Saltwater Aquarium

Capsaloidea or capsalids, including the genera Neobenedenia and Benedenia, are large oviparous monopisthocotylean flatworms (approximately 3-10 mm) commonly found in tropical saltwater aquariums.6 They may infest the skin, gills, and eyes of teleost fish and elasmobranchs, causing flashing, erratic swimming, scale loss, erosions of the skin, and ulcerations of the skin and eyes, and subsequently may lead to death. Diagnosis may be made with gross observation of the parasites, wet mount examination of the skin and gills, or histopathology. The parasites may also easily be recovered from the bottom of a bucket or tank following a freshwater dip, and this method is often used to reduce parasitic loads in heavily parasitized fish prior to further treatments. Capsalids produce many triangular-shaped eggs (more than 80 in a day in some species) that may take 4-21+ days to hatch depending on environmental temperature.6 These eggs also contain long sticky threads that are used for attachment to fish, substrate, nets, and other objects. For these reasons, they are extremely difficult to eliminate from a system once established, and efforts are usually focused on management to reduce outbreaks.

Chemical treatments are often difficult in large aquarium systems due to cost, volume, differences in species sensitivities, difficulty maintaining therapeutic levels, and bacterial degradation of the medication. Chronic exposure to reduced salinity is thought to reduce egg viability and may be a useful tool in the management of capsalids in large aquarium systems. Indefinite hyposalinity (see Table 47.1) has proven successful at preventing recurrent outbreaks of external monogeneans (Neobenedenia sp.) in a 500,000gallon mixed-species exhibit housing sea turtles, sand tiger sharks (Carcharias taurus), nurse sharks (Ginglymostoma cirratum), southern stingrays (Dasyatis americana), spiny lobsters (Panulirus argus), and teleost fish (K. Heym, personal communication, March 16, 2017). Hyposalinity has been maintained in this exhibit since 2011, with no obvious adverse effects observed in the collection species. Only when attempts were made to increase the salinity did outbreaks and resultant morbidity and mortality reoccur. Biological control with cleaner fish may also be a viable solution for reducing parasitic load in large aquarium exhibits.6

Leech Infestation in a Large Mixed-Species Saltwater Aquarium

Inadvertent introduction of *Branchellion torpedinis* leeches into closed saltwater aquariums has reported. These marine

Text continued on p. 331

TABLE Treatment Protocols for Commonly Diagnosed Parasites in Saltwater Public Aquaria

	Comments	Tx typically multimodal requiring addition of hyposalinity and/or transfer to clean aquarium and/or temperature manipulations in conjunction with chemical txs. Copper, in the form of copper sulfate, is typically the tx of choice	When restricted to external surface of fish, organisms are easily treated. When organisms invade deep muscles, internal organs, and blood vessels, there is a poor prognosis [®] Philasterides dicentrarchi and potentially Uronema spp. reportedly pathogenic in sea dragons and seahorses ^{®+25}
Ireatment Protocols for Commonly Diagnosed Farasites III Saltwater Fubric Aquaina	Treatment Options	Copper (Cu²¹) 0.15-0.2 mg/L of free Cu²⁴ for 3-6 weeks. Tx concentration should be attained slowly over 3 days ⁶ . • 25 mg/L (0.025 mL/L) EOD up to 4 weeks²7. • 125-250 mg/L (0.125-0.25 mL/L) for 60 min q 3 days ^{6,28} . Chloroquine diphosphate: • 10 mg/L prolonged bath for 2-3 weeks²7. • 10 mg/L prolonged bath for 2-3 weeks²7. • 10 mg/L prolonged bath for 2-3 weeks²7. • 10 mg/L for 21-30 days. Salinity should be reduced slowly by 5-10 g/L a day. • 15-18 g/L for 42-56 days along with temperature increases in large aquariums housing elasmobranchs, teleosts, and sea turtles may aid in resolution of infection (K. Heym, personal communication, March 16, 2017) • 10 g/L for 3 h q 3 days for 4 txs ^{8,27} . Transfer fish to clean aquarium between chemical txs or transfer to a clean aquarium q 3 days ⁸ . Temperature manipulations: Peak reproduction occurs between 20°C and 30°C requiring frequent and repeated medication dosing. Reproduction stops at 19°C°	Metronidazole: Bath at 50 mg/L daily for 10 days reportedly was successful in treating an outbreak of scuticociliatosis (Philasterides dicentrarchi) in Australian Pot-bellied Seahorses ²⁴ 50 mg/kg PO SID for 7 days followed 30 days later with ponazuril at 10 mg/kg PO SID for 3 days, and repeated in 2 weeks may have been helpful in reducing parasitic load in a zebra shark with subcutaneous scuticociliatosis (S. DiRocco, personal communication, March 2, 2017) Jenoclaen (Atacama extract 97% [Zeolites] + citric acid 3%) at 50 mg/L for 30 min to treat scuticociliatosis (Philasterides dicentrarchi) in Olive Flounder (Paralichthys olivaceus) ²⁶ Hydrogen Peroxide at 50 mg/L for 30 min to treat scuticociliatosis (Philasterides dicentrarchi) in Olive Flounder ²⁷ Formalin: 200 mg/L (0.25 mL/L) formalin bath for 2 h SID for 6 days used in Japanese flounder ⁶ 125–250 mg/L (0.125-0.25 mL/L) for 1 h may be helpful to clear early superficial skin infestation. May be used as an adjunct to a freshwater bath ²⁸ Other: Improve husbandry
nt Protocols for Commonly Diagno	Diagnosis	Wet mount of skin or gills; histopathology	Wet mount of skin, gills, or internal organs; histopathology
	Examples	Gyptocaryon irritans	Uronemia spp. Scuticociliatosis
47.1 Ireatme	Type	Protozoa: Motile Ciliates	

Secretary and	Orter an indicator or poor sanitation or overcrowding ³⁰	Often not susceptible to Cu^{2+6}	Chloroquine recently tx of choice but Cu ²⁺ still most widely used Can infest both elasmobranchs and teleosts ⁶ Clownfish seem to be particularly susceptible to this parasite ³⁰	Parasite has a direct life cycle; therefore a single tx is usually effective	Associated with morbidity and mortality in cownose rays ¹⁴	Oviparous species require multiple repeated txs (≥3) at appropriate intervals and a longer duration of tx when compared with viviparous species Reproductive rate is controlled by temperature (typically faster at warmer temperatures) ⁶
	Improve water quality and husbandry Formalin: • 25 mg/L (0.025 mL/L) 24 h bath repeated EOD for 2–3 txs if needed ^{6.28} • 125–250 mg/L (0.125–0.25 mL/L) 1-hour bath repeated SID for 2–3 days if needed ^{6.28} Cu ²⁺ at 0.15–0.2 mg/L of free Cu ²⁺ until effect ^{6.28} Others: Freshwater dip/bath	Formalin: • 125–250 mg/L (0.125–0.25 mL/L) 1 h bath repeated SID for 2–3 days if needed ⁶ • 25 mg/L (0.025 mL/L) 24 h bath repeated EOD for 2–3 txs if needed ⁶	Cu ²⁺ at 0.15–0.2 mg/L prolonged immersion for 2–3 weeks³¹ Chloroquine diphosphate at 10 mg/L prolonged immersion once or redose in 7–8 days for 2–4 txs if needed ⁶ Hydrogen Peroxide at 75 mg/L (0.21 mL of 35% H ₂ O ₂ /L) for 30 min, retreat after 6 days and immediately move fish to an uncontaminated system ⁶ Others: Temperature manipulation, hyposalinity, freshwater baths/dips	Formalin: • 25 mg/L (0.025 mL/L) 24 h bath repeated EOD if needed ^{6.28} • 125–250 mg/L (0.125–0.25 mL/L) 1 h bath repeated SID if needed ^{6.28} Metronidazole at 40 g/kg of feed at 2% BW per day for 10 days ⁶ Secnidazole at 20 g/kg of feed per day at 2% BW for at least 2 days ⁶ Triclabendazole at 40 g/kg of feed per day at 2% BW for at least 5 days ⁶ Other: Improve husbandry/sanitation	Ou ²⁺ wire particles (50 mg, Copasure) at a dose of 36.7 mg/kg administered PO once appears to be the current tx of choice ¹⁶ Toltrazuril at 10 mg/kg/day PO for 5 days may help to control but does not eliminate infection ³⁰	Praziguantel: • 2–10 mg/L immersion for 3–6 h bath, repeat in 7 days ^{6,28} • 2–3 mg/L weekly for 4–5 txs used in conjunction with Cu ²⁺ tx for oviparous monogeneans (R. George, personal communication, January 27, 2017) • 8 mg/L prolonged immersion for 20 days, redose every fourth day • 20 mg/L bath for 4 min or 20 g/kg of feed q 48 h at 1% BW were both effective in reducing polyopisthocotylea in rockfish ³² • 5 mg/L immersion for 1,800 min (30 h), 10 mg/L immersion for 120 min, or 20 mg/L for 45–90 min reportedly reduces monogenean loads on eagle rays but does not eliminate (2,18) (R. George, personal communication, February 20, 2017)
	Wet mount of skin or gills; histopathology	Wet mount of skin or gills; histopathology	Wet mount of skin or gills; histopathology	Wet mount of skin or gills; histopathology	Wet mount of coelomic saline flush; histopathology	Wet mount of skin or gills; gross visual observation of large monogeneans; histopathology
	Trichodina spp.	Brooklynella spp.	Amyloodinium ocellatum	Ichthyobodo spp.	Eimeria southwelli	Monopisthocoty- leans Gyrodactylidae (viviparous) Dactylogyridae (oviparous) Capsalidae (oviparous) Monocotylidae (oviparous) Polyopisthocotylea (oviparous)
			Protozoa: External Dinoflagellate	Protozoa: External Flagellate	Protozoa: Coccidia	Metazoan: Monogeneans

Continued

Treatment Protocols for Commonly Diagnosed Parasites in Saltwater Public Aquaria—cont'd

47.1

more resistant to treatment an affinity for gill tissue. Gill Capsalids have an affinity for Praziquantel is generally the frequency of repeated or monogeneans are often Polyopisthocotylea have Dactylogyroidea are most in the water to confirm being maintained. This to measure drug levels be used to decide the tx of choice; however, therapeutic levels are knowledge may then it is strongly advised commonly found in Dacylogyrids and freshwater fish ocular tissue redosed txs Comments Indefinite hyposalinity (20-24 ppt) has proven successful at preventing recurrent 100 mg/kg BW via oral gavage reportedly reduces monogenean loads in eagle with 10 mg/L chloroquine has been utilized successfully by at least one facility detected after 2 months of treatment. Chloroquine concentration should begin also recommended to remove ozone from the treatment tanks because it may mixed species exhibit (500,000 gallons) housing teleosts, elasmobranchs, and an adjunct to praziquantel (3 mg/L weekly for 4-5 txs). Has been used without of 10 mg/L is achieved. No obvious adverse effects were noted in eagle rays Treatment concentration should be attained slowly over 3 days. Often used as react with the chloroquine and cause inappetance or other adverse effects in changes in swimming behavior during the treatment and were removed. It is adverse effects in cownose rays but has caused inappetence in southern and 0.25-1 mg/L prolonged immersion for 2 tx at 3-day intervals for dactylogyrid treating with 3 mg/L praziquantel q 5 days for 4 to 5 months in conjunction at 3 mg/L and slowly increase by 2-3 mg/L EOD until a final concentration species. Has been used as an adjunct treatment prior to praziquantel use. outbreaks of external monogenean (Neobenedenia sp.) parasites in a large Cu²⁺ 0.15 mg/L of free Cu²⁺ for 3-4 months to treat oviparous monogeneans. to treat monogeneans in eagle rays such that all life stages ceased to be elasmobranchs (R. George, personal communication, February 20, 2017). Atlantic stingrays (R. George, personal communication, January 27, 2017) 5 mg/L praziquantel prolonged immersion for 1,800 min (30 h) in a naïve or Atlantic stingrays at this facility; however, cownose rays demonstrated treatment tank, moving animals to a second naïve treatment tank, then rays but does not eliminate. More research is currently underway? sea turtles (K. Heym, personal communication, March 16, 2017) 15-25 mg/L (0.015-0.025 mL/L) prolonged immersion^{6.28} <20 g/L may reduce egg viability in oviparous species⁶ 150-250 mg/L (0.15-0.25 mL/L) bath for 60 min^{6.28} 100 mg/kg BW split into four doses SID for 3 days⁶ 2–5 mg/L bath for 60 min⁶ 100 mg/L bath for 10 min⁶ **Treatment Options** Trichlorfon (Dylox): Mebendazole: Hyposalinity: Formalin: Diagnosis Examples Type

Biological control: French angel fish, neon gobies, cleaning gobies, and blue-lined

1 mg/L prolonged immersion for 24 h⁶

Other: Freshwater dip/bath to reduce parasite load, chloramine T

cleaner wrasse pick monogeneans off other fishess

Most common in freshwater wild fish. Uncommon in aquaria due to the complex life cycle involving a variety of host animals Self-limiting and does not progress in aquariums without exposure to hosts	Tx targets adults not larvae Do not feed live foods that might transmit larval cestodes	Tx targets adults, not larvae. Encysted nematodes are difficult to treat. Fish may be definitive hosts for adult nematodes or act as an intermediate host or transport for larval nematodes. Most nematodes are oviparous, but there are some that are viviparous and some with direct life cycles. Ivermectin has been used but is not recommended due to low therapeutic index ³⁰	Premedication with atropine at 0.04–0.08 mg/kg IM is advised (A. McDermott, personal communication, January 26, 2017) Spotted eagle rays anecdotally appear more sensitive to this treatment; therefore, pre-medication with 20.06 mg/kg of atropine is highly advised Organophosphates are typically the tx of choice
Prevention: exclude birds and mammals from contact with fish, disinfect and quarantine, intermediate host usually mollusk. Praziquantei: 1 mg/L immersion for 90 h ⁶ 2-10 mg/L for 24 h prolonged immersion ⁶ 10 mg/L bath for 1 h ⁶ 25 mg/kg BW IM once ⁶ 50 mg/kg BW PO once ⁶ 330 mg/kg BW PO once ⁶ Other: Cu ²⁺ , Slaked lime, and Bayluscide molluscicides	Praziquantel: • 5 mg/kg BW PO q 7 days up to 3 txs ²⁸ • 50 mg/kg BW PO once ⁸ • 2 mg/L bath for 1–3 h, repeat in 1 week if needed ⁸ Other: Exclude intermediate host contact with fish and water supply	Fenbendazole: 2.5 mg/L once a week for 3 weeks ^{6,28} 2.5 mg/g of food daily for 3 days, repeat in 2–3 weeks ²⁸ 5.6 mg/kg BW PO for 3 days ⁶ 5.0 mg/kg BW PO once a week for 2 txs ⁶ Levamisole: 4 g/kg of food once a week for 3 weeks ²⁸ 2.5–10 mg/kg BW PO once, then repeated in 14 days resolved <i>Huffmanela</i> sp. lesions in sandbar sharks ²⁴ 10 mg/kg BW PO once, then repeated at 14 and 28 days. Cleared <i>Huffmanela</i> sp. lesions in sandbar shark (<i>Carcharhinus plumbeus</i>) ³⁴ 10 mg/kg BW IM once, repeated at 14 and 28 days. Cleared <i>Huffmanela</i> sp. egg tracks from a sandbar shark (<i>Carcharhinus plumbeus</i>) ³⁴ 10 mg/L bath for 3 days ^{28,30} 11–2 mg/L immersion for 24 h, repeat in 2–3 weeks ²⁸ 10 mg/L bath for 3 days ^{28,30} Other: Piperazine, trichlorphon, and mebendazole. Avoid feeding organisms to fish that may harbor the larvae (e.g., live copepods are a common source)	Trichlorfon (Dylox) at 0.25–0.4 mg/L for a 5–6 h bath was used successfully to treat Branchellion torpedinis leeches in elasmobranchs. Repeated tx in 30 days may be needed (A. McDermott, personal communication, January 26, 2017). It is important to remember when calculating the dose that commercial preparations of organophosphates vary in percentage of active ingredients ⁶
Wet mounts of gill, skeletal muscle, intestinal contents, or other organs; histopathology	Wet mount of intestinal content or other internal tissues/ organs; histopathology	Wet mount of feces, intestinal contents, or other internal tissues/ organs; histopathology	Gross visual observation or wet mount of skin, gills, and oral cavity samples; histopathology
Metazoan: Digeneans	Metazoan: Cestodes	Metazoan: Nematodes	Metazoan: Leeches (Annelids)

TABLE Treatment Protocols for Commonly Diagnosed Parasites in Saltwater Public Aquaria—cont'd

	Comments	Diflubenzuron is considered the most effective tx for crustacean parasites ³⁰ Enamectin has been associated with rare neurotoxicity and death when a fish ingests a greater amount of food than anticipated. Reduce the dose for ravenous fish (L. Adams, personal communication, March 14, 2017) It is important to remember when calculating trichlorfon doses that commercial preparations often vary in percentage of active ingredients ⁶ Carbaryl treatment may cause anorexia and at a dose of 0.5 mg/L has resulted in muscle tetany or spasms in fish but has resolved 24 h following removal of the medication (L. Adams, personal communication, March 14, 2017)
	Treatment Options	Diffubenzuron: 0.01 mg/L immersion for 48 h q 6 days for 3 txs²8 0.03 mg/L immersion for Lemaea spp.³ 75 mg/kg BW PO for 14 days for sea lice® Enamectin: • 50 µg/kg BW PO for 7 days for sea lice® Enamectin: • 50 µg/kg BW PO for 7 days for sea lice® 2 mg per 100 g of dry gel food, add water, and mix to create a medicated gel. Feed approximately 2% BW for an estimated fish intake of 400 µg/kg/day for 10 days for treatment of copepods (L. Adams, personal communication, March 14, 2017) Trichlorfon: • 15–300 mg/L for 15–60 min at 3–18°C for sea lice® • 2–5 mg/L for 80 min for isopods® • 0.25–1 mg/L immersion® Dichlorvos: • 0.5–2 mg/L for 30–60 min for sea lice® • 15 mg/L for 1 min for sea lice® • 15 mg/L for 1 min for sea lice® • 15 mg/L for 1 min for sea lice® • 15 mg/L for 1 min for sea lice® • 15 mg/L for 1 min for sea lice® • 15 mg/L for 1 min for sea lice® • 15 mg/L for 1 min for sea lice® • 15 mg/L for 1 min for sea lice® • 15 mg/L for 1 min for sea lice® • 10.25–0.35 mg/L for 2 tx sat 48 h intervals with water changes in between (L. Adams, personal communication, March 14, 2017) Milbemycin oxime (Interceptor) alone or with spinosad (Triflexis) has been used for treatment of copepods: • 0.25–1 mg/Rg PO once • 4 mg/100 g of gel food (0.004%) fed one day (L. Adams, personal communication, January 27, 2017) Other: Manual removal, biological control, and freshwater dip/bath
	Diagnosis	Gross visual observation or wet mount of gills, skin, or oral cavity samples; histopathology
	Examples	Copepods Branchlurans Isopods
1./4	Type	Metazoans: Crustaceans

The listed doses have been acquired from both published and anecdotal references and may not be appropriate for all species in all water conditions. Many of the listed treatments are not yet supported with pharmacokinetic data; therefore the reader is encouraged to use caution. If adverse reactions are observed, fish should be removed from the treatment immediately. BW, Body weight; EOD, every other day; h, hour(s); IM, intramuscular, min, minutes; PO, orally; q, every; SID, once daily; tx(s), treatment(s).

leeches exclusively parasitize elasmobranchs, resulting in ulcerations at attachment sites, lethargy, anorexia, anemia, and potential death in as little as 5 days (A. McDermott, personal communication, January 26, 2017).6,11 Leeches may also serve as vectors for infectious diseases. Affected species have included sawfish (Pristis pristis), guitarfish (Rhina ancylostoma), zebra sharks (Stegostoma fasciatum), spotted eagle rays (Aetobatus narinari), manta rays (Manta birostris), southern stingrays, and experimentally vellow stingrays (Urobatis jamaicensis) (A. McDermott, personal communication, January 26, 2017).11 Leeches are most commonly recovered from the claspers, pectoral fins, eyes, oral cavity, and cephalic lobes and appear to remain permanently attached to the host if not removed. Manual removal when leeches are easily accessible is a treatment option, but the process is time consuming. Trichlorfon (Dylox) for a 5- to 6-hour bath has also been used with success; however, it is strongly recommended to premedicate with atropine approximately 45-60 minutes prior to trichlorfon treatment (see Table 47.1). It is also important to remember when calculating the dose that commercial preparations of organophosphates vary in percentage of active ingredients.6 Leech cocoons will hatch in approximately 30 days; therefore repeated treatment may become necessary at that time.11 Topical or systemic antibiotic administration and blood transfusions have also been helpful in the supportive care and recovery of the affected host when deemed necessary by the veterinarian. Future studies to establish pharmacokinetic effects and safety for trichlorfon in various species of elasmobranchs are warranted.

Monogeneans in Spotted Eagle Rays (Aetobatus narinari)

Decacotyle floridana and Clemacotyle australis are monocotylid monogenean parasites that have been associated with morbidity and mortality in spotted eagle rays. 5,12,13 These parasites have been recovered from the skin and gills of spotted eagle rays, and clinical signs have included abnormal swimming postures, bottom resting, and rubbing on the walls of the enclosure. 12 Praziquantel immersion (see Table 47.1) reportedly reduces parasite loads and is often used as a management tool; however, this treatment does not successfully eradicate the parasite, requires repeated therapy and frequent handling, and is further complicated by bacterial degradation of the medication in the water. 12,13 A multistep treatment protocol involving a prolonged immersion of praziquantel in a naïve treatment tank for 30 hours followed later by immersion with chloroquine in combination with praziquantel for 4-5 months in a second naïve treatment tank (see Table 47.1) was utilized successfully by at least one facility to treat monogeneans in eagle rays such that all life stages ceased to be detected. No obvious adverse effects were noted in eagle rays or Atlantic stingrays with this treatment protocol at this facility; however, cownose rays demonstrated changes in swimming behavior during this treatment and were removed. It is also recommended to remove ozone before treatment because it is believed to react with chloroquine and cause inappetance or other adverse effects in elasmobranchs (R. George, personal communication, February 20, 2017). Praziquantel administered via gastric gavage to anesthetized spotted eagle rays has also resulted in dramatic decreases in parasite loads but did not eliminate (see Table 47.1).⁵ Research investigating pharmacokinetic data for this oral treatment regimen and various other chemical immersions along with additional management options are currently ongoing.

Eimeria southwelli in Cownose Rays (Rhinoptera bonasus)

Eimeria southwelli are apicomplexa coccidia parasites that may be associated with morbidity and mortality in cownose rays at high numbers. 14 In small numbers and in the absence of clinical signs, this parasite might be considered normal flora that may clear over time without treatment. 15 Clinical signs have included discoloration of the skin, emaciation, and death. Diagnosis includes microscopic identification of organisms on a wet mount obtained from a coelomic saline flush. A coelomic flush may be obtained in one of two ways: (1) gently passing a lubricated sterile red rubber catheter (3-5 Fr) attached to a syringe through one of the coelomic pores found on either side of the cloaca or (2) inserting a winged infusion set attached to a syringe (21-gauge, 19-mm needle) into the right ventral paramedian body wall cranial to the pelvic girdle. Sterile 0.9% saline may be infused into the coelomic cavity at 1% of the animal's body weight and aspirated for microscopic examination.¹⁴ The current most successful treatment when clinical signs are apparent appears to be copper wire particles (Copasure) administered orally once (see Table 47.1).16 No obvious negative effects have been documented to date, and the animals do not appear to excrete copper to detectable levels in their enclosure following treatment (E. Clarke, personal communication, January 26, 2017). Further studies to establish pharmacokinetic effects and safety for copper wire particles in elasmobranchs are needed. Toltrazuril, ponazuril, clindamycin, and sulfadimethoxine treatments administered at various doses, frequencies, and routes have been attempted with inconsistent or inconclusive results. A preliminary study evaluating the use of ponazuril in cownose rays did not achieve blood concentrations considered to be therapeutic in other species, but the parasite load did decrease and there were no negative side effects observed (S. Cassle, personal communication, January 24, 2017). Cloacal prolapse may be a complication of the parasitic infection and/or treatment attempts associated with the infection.17

Copper Immersion in Cownose Rays (Rhinoptera bonasus)

The use of copper immersion treatment has typically been avoided in elasmobranchs due to intolerance and mortalities

associated with respiratory, osmoregulatory, and ionoregulatory distress. 3.15.18-21 However, it has been used safely as a prolonged immersion in cownose rays for 3–4 months in conjunction with praziquantel prolonged immersion weekly for 4–5 treatments (see Table 47.1) to treat capsalid parasite infestations (R. George, personal communication, January 27, 2017). Closely monitoring copper and praziquantel concentrations in the water is extremely important throughout the course of treatment. Although no adverse effects have been observed in cownose rays, it has been associated with inappetence in southern and Atlantic stingrays (Dasyatis sabina) (R. George, personal communication, January 27, 2017). Pharmacokinetic studies to evaluate the effects and safety of copper immersion at various concentrations in elasmobranchs are warranted.

Scuticociliatosis in Sygnathid and Elasmobranch Species

Philasterides dicentrarchi are ciliated protozoa in the subclass Scuticociliatia that have been identified as the cause of disease outbreaks in a range of marine teleost fish in aquarium and aquaculture settings.²² Severe outbreaks of this parasite have recently been reported in aquarium-maintained Australian pot-bellied seahorses (Hippocampus abdominalis), weedy sea dragons (Phyllopteryx taeniolatus), and leafy sea dragons (Phycodurus eques) and may be associated with stressful environmental conditions (e.g., temperature fluctuations, poor water quality, transport) resulting in compromise of the immune system. Clinically these animals have presented with nodular or ulcerative epidermal lesions, hyperemia or depigmentation, anorexia, lethargy, irregular respirations, abnormal swimming, and/or death. Histopathologic examination revealed ciliate invasion into the gills, dermis, subdermal connective tissues, vasculature, skeletal muscle, ovary, kidney, intestines, thyroid, and/or brain.²³⁻²⁵ Treatment with a prolonged immersion of metronidazole (see Table 47.1) for 10 days appeared to be successful in treating systemic disease in Australian pot-bellied seahorses; however, knowledge of additional treatment options for sygnathids is lacking in the literature, and further pharmacokinetic research is warranted.24

Philasterides dicentrarchi has also been the cause of a rapidly lethal systemic infection in aquarium-maintained zebra sharks, Port Jackson sharks (Heterodontus portusjacksoni), and Japanese horn sharks (Heterodontus japonicus) characterized by necrotizing hepatitis, meningoencephalitis, and/or thrombosing branchitis. Clinical signs prior to death were brief and included lethargy, anorexia, and/or behavioral abnormalities. ²² Ciliate infections resembling scuticociliates have also been identified in swell sharks (Cephaloscyllium ventriosum), dusky smooth-hounds (Mustelus canis), southern stingrays, and California bat rays (Myliobatis californica) (C. Erlacher-Reid, unpublished, 2016). ^{22,26} These infections have varied in severity, ranging from external infections of the gills and skin, along with bacterial, flagellate, or viral infections, to deep invasive lesions involving the kidney,

brain, and/or liver. It is thought that this disease may have been underreported or unrecognized in elasmobranchs until now or is an emerging disease. Systemic invasion has been associated with a poor prognosis. To date, there are no known effective treatment protocols published in elasmobranchs. However, treatment with oral metronidazole followed a month later with oral ponazuril may have been helpful in clearing or reducing parasitic load in a zebra shark with subcutaneous scuticociliatosis (see Table 47.1). Follow-up on the case to monitor for recurrence is currently ongoing (S. DiRocco, personal communication, March 2, 2017).

Summary

Prevention and early diagnosis of parasitic infections are recommended by establishing a thorough quarantine and preventative medicine protocol whenever possible. There are a multitude of published and unpublished protocols that have been used for the treatment and management of parasitic infections in fish with variable success; therefore the reader is strongly encouraged to use caution and perform a thorough literature search and discuss treatment options with experienced colleagues to select the treatment protocol that is best suited for each specific situation (see Table 47.1). There are numerous considerations that should be addressed prior to and during treatment, including potential species-specific sensitivities, life cycle of the parasite, and the relationship between and among water quality parameters, parasites, fish, and treatments. The reader is encouraged to monitor drug concentrations throughout treatment to determine appropriate dosage frequencies and ensure therapeutic levels are maintained. Further pharmacokinetic research evaluating the effects and safety of antiparasitic medications in various species of fish are greatly needed in the available peer-reviewed literature. The World Association for the Advancement of Veterinary Parasitology (WAAVP) has created guidelines for testing the efficacy of ectoparasiticides in finfish, and this may serve as a useful resource for designing meaningful studies.35

References

- Harms CA: Treatments for parasitic diseases of aquarium and ornamental fish, Sem Avian Exotic Pet Med 5:54

 –63, 1996.
- Harms CA: Fish. In Fowler ME, Miller RE, editors: Zoo and wild animal medicine, ed 5, St. Louis, MO, 2003, Elsevier, pp 2–20.
- Hadfield CA, Clayton LA: Fish quarantine: current practices in public zoos and aquaria, J Zoo Wildl Med 42:641–650, 2011.
- Hadfield CA: Quarantine of fish and aquatic invertebrates in public display aquaria. In Miller RE, Fowler ME, editors: Zoo and wild animal medicine: current therapy (vol 7). St. Louis, 2012, Elsevier, pp 202–209.
- MyIniczenko ND, Nolan EC, Thomas A, et al: Management of monogenes in eagle rays (Aetobatus narinari) with high dose oral praziquantel, in Proceedings of the 48th annual IAAAM Conference, 2015.
- Noga EJ: Fish disease: diagnosis and treatment, ed 2, Ames, IA, 2010, Wiley-Blackwell.

- Larrat S, Marvin J, Lair S: Low sensitivity of antemortem gill biopsies for the detection of subclinical *Pseudodactylogyrus bini* infestations in American eels (*Anguilla rostrata*), *J Zoo Wildl Med* 43:190–192, 2012.
- Knight SJ, Boles L, Stamper MA: Response of recirculating saltwater aquariums to long-term formalin treatment, J Zoo Aquar Res 4:77–84, 2016.
- Thomas A, Dawson MR, Ellis H, et al: Praziquantel degradation in marine aquarium water, *PeerJ* 4:e1857, 2016, doi:10.7717/ peerj.1857.
- Kuemmerer K: Antibiotics in the aquatic environment: a review. Part II, Chemosphere 75:435

 –441, 2009.
- Marancik DP, Dove AD, Camus AC: Experimental infection of yellow stingrays (*Urobatis jamaicensis*) with the marine leech (*Branchellion torpedinis*), Dis Aquat Organ 101:51–60, 2012.
- Janse M, Borgsteede FHM: Praziquantel treatment of captive white-spotted eagle rays (Aetobatus narinari) infested with monogean trematodes, Bull Eur Ass Fish Pathol 23:152–156, 2003.
- Nolan EC, MyIniczenko ND, Steinmetz J, et al: Oral praziquantel pharmacokinetics in spotted eagle rays (Aetobatus narinari), in Proceedings of the 47th annual IAAAM Conference, 2016.
- Stamper MA, Lewbart GA: Eimeria southwelli infection associated with high mortality of cownose rays, J Aquat Anim Health 10:264–270, 1998.
- MyIniczenko ND. Medical management of rays. In Miller RE, Fowler ME, editors: Zoo and wild animal medicine: current therapy (vol 7). St. Louis, 2012, Elsevier, pp 170–176.
- Clarke EO, Grzenda K, Murphy D, et al: Successful treatment of Eimeria southwelli in a cownose ray (Rhinoptera bonasus) using oral copper wire particles, in Proceedings of the 46th annual IAAAM Conference, 2016.
- McDermott A, Field C, Mejia-Fava J, et al: Medical and surgical management of cloacal prolapses in cownose rays (*Rhinoptera bonasus*) undergoing treatment for *Eimeria southwelli* infection, in Proceedings of the 44th annual IAAAM Conference, 2013.
- Boeck GD, Hattink J, Franklin NM, et al: Copper toxicity in the spiny dogfish (Squalus acanthias): urea loss contributes to the osmoregulatory disturbance, Aquat Toxicol 84:133–141, 2007.
- Grosell M, Wood CM, Walsh PJ: Copper homeostasis and toxicity in the elasmobranch Raha erinacea and the teleost Myoxocephalus octodecemspinosus during exposure to elevated water-borne copper, Comp Biochem Physiol C Toxicol Pharmacol 135:179–190, 2003.
- Grosell M: Intestinal anion exchange in marine fish osmoregulation, J Exp Biol 209:2813–2827, 2006.
- Grosell M, Blanchard J, Brix KV, et al: Physiology is pivotal for interactions between salinity and acute copper toxicity to fish and invertebrates, Aquat Toxicol 84:162–172, 2007.
- Stidworthy MF, Garner MM, Bradway DS, et al: Systemic scuticociliatosis (*Philasterides dicentrarchi*) in sharks, Vet Pathol 51:628–632, 2014.

- Bonar CJ, Garner MM, Weber ES, et al: Pathological findings in weedy (*Phyllopteryx taeniolatus*) and leafy (*Phycodurus eques*) seadragons, Vet Pathol 50:368–376, 2013.
- Cicco ED, Paradis E, Stephen C, et al: Scuricociliatid ciliate outbreak in Australian pot-bellied seahorse, *Hippocampus* abdominalis (Lesson, 1827): clinical signs, histopathologic findings, and treatment with metronidazole, *J Zoo Wildl Med* 44:435–440, 2013.
- Rossteuscher S, Wenker C, Jermann T, et al: Severe scuticociliate (*Philasterides dicentrarchi*) infection in a population of sea dragons (*Phycodurus eques* and *Phyllopteryx taeniolatus*), Vet Pathol 45:546–550, 2008.
- Garner MM: A retrospective study of disease in elasmobranchs, Vet Pathol 50:377–389, 2013.
- 27. Yanong RPE: Cryptocaryon irritans infections (marine white spot disease) in fish. Program in Fisheries and Aquatic Sciences, SFRC, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL, 2009. Retrieved 1 March 2017 from http://edis.ifas.ufl.edu/ fa164.
- Lewbart GA: Fish. In Carpenter JW, editor: Exotic animal formulary, ed 4, St. Louis, MO, 2013, Elsevier, pp 18–52.
- Jin C-N, Harikrishnan R, Moon Y-G, et al: Effectiveness of chemotherapeutants against scuticociliate *Philasterides dicentrarchi*, a parasite of olive flounder, *Vet Parasitol* 168:19–24, 2010.
- Petty BD, Francis-Floyd R: Parasitic diseases of fish. In Aiello SE, Moses MA, editors: *The Merck veterinary manual*, ed 11, NJ, 2016, Merck & Co., Inc.
- Reed P, Francis-Floyd R: Amyloodinium infections of marine fish.
 College of Veterinary Medicine, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL, 1994. Retrieved 1 March 2017 from http://agrilife.org/fisheries/files/2013/09/Amyloodinium-Infections-of-Marine-Fish.pdf.
- Kim KH, Cho JB: Treatment of Microcotyle sebastis (Monogenea: Polyopisthocotylea) infestation with praziquantel in an experimental cage simulating commercial rockfish Sebastes schlegeli culture conditions, Dis Aquat Organ 40:229–231, 2000.
- Cowell LE, Watanabe WO, Head WD: Use of tropical cleaner fish to control the ectoparasite Neobenedenia melleni (Monogenea: Capsalidae) on seawater cultured Florida red tilapia, Aquaculture 113:189–200, 1993.
- MacLean RA, Fatzinger MH, Woolard KD, et al: Clearance of a dermal Huffmanela sp. in a sandbar shark (Carcharhinus plumbeus) using levamisole, Dis Aquat Organ 73:83–88, 2006.
- Sommerville C, Endris R, Bell TA, et al: World association for the advancement of veterinary parasitology (WAAVP) guideline for testing the efficacy of ectoparasiticides for fish, Vet Parasitol 219:84–99, 2016.