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Using Garlic (Allium sativum) as a Masking Agent to Improve Palatability of Praziquantel-Medicated Feed for Juvenile Yellowtail Kingfish (Seriola lalandi)

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By

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The delivery of praziquantel in feed has the potential to be both an economically and logistically feasible method of treating monogenean ectoparasitic infections in *Seriola* spp. if the associated palatability issues can be overcome. Praziquantel treatment has been proven effective in previous efficacy trials against *Benedenia seriolae* and *Zeuxapta seriolae*, reducing the overall stress levels and potential mortality in hatchery and sea cage environments, however palatability issues currently constrain its commercial use. This study aimed to examine the potential of garlic paste (GB1) as a masking agent for praziquantel feed inclusion by surface coating both control and medicated pellets at differing concentrations. Acclimation feeds were delivered and a daily feed ration of 10g was selected in order to observe approximately 100% consumption in the control group. The results of this palatability trial indicate that surface coating GB1 at a rate of 50ml/kg significantly improves the palatability of a diet containing 5g/kg of praziquantel, while inclusion at a rate of 20ml/kg was ineffective at improving palatability.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

Global aquaculture production as of 2013 is 66 million metric tons (MMT), nearly 40% of all total world fishery production, at an approximate value of US$119 billion. Aquaculture has surpassed global beef production (63MMT) and now provides greater than 50% of all seafood for human consumption. One of the primary finfish genuses cultured is *Seriola* spp. (Carangidae). In 2010, total production of this genus was close to 200,000 tons, second only to unidentified marine species (FAO, 2013). The monogenean parasite species *B. seriolae* and *Z. seriolae*, however, have had significant impacts on the *Seriola* spp. industries that they affect (Benetti et al., 2005). Whittington et al. (2001) reports that the Japanese yellowtail industry (which produces a combination of *S. quinqueradiata, S. dumerili* and *S. lalandi*), produced approximately 150,000 tons in 2011, however up to 20% losses in total production are attributed to disease (FAO, 2013). Ernst et al. (2005) estimates that, when including treatment cost, reduced growth and feed conversion rate (FCR) and mortality, managing ectoparasite outbreaks can account for up to 20% of the total production cost. It is further estimated that nearly US$230 million is spent on medication for all *Seriola* spp. related diseases annually (Sharp et al., 2001 as cited in Whittington and Chisholm, 2008).

1.1 Monogenean Ectoparasites

Benedenia seriolae (Yamaguti 1934, Sub-class Monopisthocotylea) and *Zeuxapta seriolae* (Meserve 1938, Sub-class Polyopisthocotylea), gill and skin flukes respectively, commonly infect the genus *Seriola* (Rohde, 1978; Benetti et al., 2005). The monopisthocotylean *B. seriolae* are flattened dorsoventrally and "leaf-shaped," with little

pigmentation rendering them nearly transparent. They feed on the host's mucus and epithelial cells by attaching themselves using "anterior adhesive pads and a posterior opisthaptor" that pierces the skin (Ellis et al., 1989 as cited in Sharp et al., 2003). The individual parasites range from 4-12mm long and 1-6mm across (Whittington and Chisholm, 2008). *B. seriolae* release eggs individually and continuously as a strategy to find suitable hosts for attachment (Mooney et al., 2008). The eggs float in the water column and are carried by oceanic currents until attaching to a surface or sinking to the bottom (Chambers and Ernst, 2005). The eggs are seasonally affected, requiring more time to develop in lower temperatures (5-day embryonation at 24-28°C as opposed to 19 days at 14°C) and have an optimum salinity range of 25-35ppt (hatching delayed up to 20% at 20ppt and 60% at 50ppt) (Ernst et al., 2005). The subsequent larvae have been observed to survive for approximately 1 day unless a host organism is found, and take about 14 days to reach sexual maturity at 23°C (Hoshina, 1968 as cited in Chambers and Ernst, 2005; Kearn et al., 1992). Tubbs et al. (2005) further elaborated that at 13°, 18° and 21°C it took 48, 25 and 20 days, respectively, for parasites on *Seriola lalandi* to mature.

Fish infected with *B. seriolae* are often observed rubbing against the sides of the tank or enclosure, leading to external damage that can be further infected by other opportunistic bacteria (Sepúlveda and Gonzalez, 2014). Whittington and Chisholm (2008) describe the chronology of symptoms on *Seriola lalandi* as follows: "flashing behavior," dark spots on the epithelium, reduction in growth and FCR, lesions, loss of appetite, secondary infections, and the potential for mortality.

Z. seriolae, a polyopisthocotylean monogenean, is 4-10 mm in length and has a similar posterior opisthaptor, a feature unique to monogeneans (Whittington and Chisholm, 2008) and anterior oral suckers used to attach to the gill filaments in a "leech-like" manner in order to feed on the blood, a parasitosis known as "zeuxaptosis" (Sharp et al., 2003; Mooney et al., 2006; Grau et al., 2003). This species has exhibited a predictable egg-laying rhythm, producing nearly three quarters of their daily egg total within the first three hours after dusk, coinciding with the diurnal behavior of wild kingfish and therefore increasing the probability of attaching to a host organism. The eggs are released as filamentous "strings," which is likely a strategy to increase the chances of finding a suitable host as well. Like *B. seriolae*, *Z. seriolae* exhibit an inverse relationship between host deposition and initial egg release time. *Z seriolae*, however, are capable of releasing up to 10 times more eggs over a 24-hour period than *B. seriolae* (Tubbs et al., 2005). On *Seriola lalandi* in Australia, *Z. seriolae* took about 52, 37, and 25 days to reach sexual maturity at 13°, 18° and 21°C, respectively (Tubbs et al., 2005). Symptoms of "zeuxaptosis" infections include lethargic behavior, an emaciated or anemic appearance due to appetite loss, hyperplasia of the gill epithelium, and loss of gill color, while more serious infections have resulted in mortality due to asphyxiation (Mooney et al., 2006, Whittington and Chisholm, 2008). This is because the parasites cause lamellar lesions and block water and gas exchange via fluke, egg and filament entanglement within the gills (Grau et al., 2003). The haematocrit values are also typically lower in fish infested with this parasite (Montero, 2004).

Sharp et al. (2003) noted the presence of the monogenean ectoparasites *Z. seriolae* and *B. seriolae* in wild-caught yellowtail kingfish (*Seriola lalandi*) off the coast of New

Zealand, along with *Caligus lalandei*, *Caligus aesopus*, *Neobrachiella* spp. and *Lernanthropus* spp., all copepod ectoparasites. While the numbers of each of these species was variable among the wild samples, the monogeneans were the only species found on all of the samples. The presence of these ectoparasites on wild fish is significant because they will be introduced into hatcheries and aquaculture operations unless appropriate quarantine protocols are followed (Sharp et al., 2003).

1.2 Parasitic Threats

These monogenean ectoparasites pose a serious pathogenic threat for aquaculture in high-density environments for several reasons: the high stocking densities enhance the parasite's ability to spread, and factors such as water quality and potential nutritional deficiencies in artificial diets may have a negative impact on the fish's immune response (Whittington and Chisholm, 2008). Another concern is that monogenean parasites have a single host life cycle, requiring no intermediate host for survival (Rohde, 1993 as cited in Tubbs et al., 2005). Sea cages have other potential infections sources as the eggs often become entrapped in the sea cage netting or in the associated bio-fouling accretion due to their filamentous structure, accumulating there and effectively surrounding the fish (Mooney et al., 2006; Ogawa, 2002). This issue has been observed internationally in *Seriola* spp. cage culture, with *B. seriolae* observed on *Seriola quinqueradiata* in Japan, *Z. seriolae* on *Seriola dumerili* in the Mediterranean and both monogeneans found on *Seriola lalandi* in Australia and New Zealand (Tubbs et al., 2005; Sharp et al., 2003; Benetti et al., 2005). Up to 64,000 *B. seriolae* eggs have been observed per square meter of cage material in Japan (Ernst et al., 2002). Hutson et al. (2003) found that there was an

"extreme" likelihood of encountering both species of monogenean parasite in *Seriola* spp. operations in Australia, with high-level consequences for *B. seriolae* and moderate for *Z. seriolae*. Chambers and Ernst (2005) elaborated on the issue of infection and re-infection in sea cage farms, stating that cage layout often leads to higher re-infection rates. Løland (1993) suggested that "neutrally buoyant contaminants" remain concentrated in the wake of cages, and as *B. seriolae* larvae are only able to swim at 14.4-40 m/h, they would be dispersed according to local currents. Sea cages oriented in line with the current are therefore more susceptible to re-infection due to *B. seriolae* transported from upstream cages. Cages are frequently oriented in line with the current, however, because it facilitates mooring system design, daily operations, and helps to maintain net-pen shape. *Z. seriolae* dispersion may be limited because the eggs are released attached to filaments, however both species could have increased dispersion at lower temperatures because the eggs require more time to develop before hatching (Chambers and Ernst, 2005).

1.3 Treatments

Effective treatment for ectoparasitic infections requires full knowledge of the parasite's life cycle and the effect of external environmental parameters, as treatments that disrupt the life cycle are more likely to be successful and to minimize re-infection. The host fish will be exposed to fewer infectious stage parasites and fewer treatments will be necessary, which is beneficial both biologically and financially. In many cases this involves integrated treatments plans suitable for the local conditions (Tubbs et al., 2005).

1.3.1 Bath Treatments

One of the first treatments against monogenean ectoparasites was demonstrated in Japan for the *Seriola quinqueradiata* commercial farming industry during the late 1960's. Tributyl tin oxide (TBTO), an antifouling paint, was thought to be toxic to *B. seriolae* larvae and potentially the eggs as well, however the chemical was banned in 1990 due to environmental contamination concerns (Whittington et al., 2001). Copper sulfate, potassium permanganate, sodium peroxycarbonate, sodium chloride and trichlofon have also been used to treat monogenean infections in fish, however toxicity concerns with these chemicals require extreme precision to be approved for use with food fish (Thoney and Hargis, 1991).

Hydrogen peroxide and formalin baths are the most common chemicals used to treat monogenean infections in sea cages. They are effective in treating the parasites on the animals, but the fish are susceptible to re-infection upon reentering the cage due to eggs retained in the mesh netting (Ernst, 2005). These treatments may also be ineffective at killing the eggs in the netting or the associated biofouling (Sharp et al., 2004). Tubbs et al. (2005), however, describes how secondary treatments, if timed correctly, can interrupt the ectoparasites' life cycles. He states that at 21°C the secondary treatment should occur 11-20 days after the initial, as opposed to 13-25 days at 18°C and 24-48 days at 13°C (Tubbs et al., 2005).

Sharp et al. (2004) further elaborates on the use of formalin and Aqui-S, a clove oil based anesthetic as treatment options. Formalin has been approved for use in aquaculture by the US Food and Drug Administration (FDA) for the treatment of ectoparasites such as *B. seriolae* and *Z. seriolae*. Aqui-S, while commonly used as an

anesthetic to allow for gentle handling of fish, has also been examined as a potential treatment because of a trial by Svendson and Haug (1991) where benzocaine, another anesthetic, was found to cause monogenean parasites to detach from their hosts. Aqui-S is more effective than other anesthetics (benzocaine and MS-222) at lower temperatures and demands less recovery time after use (Stehly and Gingerich, 1999). Despite this, Sharp et al. (2004) found Aqui-S to be nearly ineffective at removing both *B. seriolae* and *Z. seriolae* from infected kingfish (*Seriola lalandi lalandi*). The Aqui-S also failed to prevent egg incubation by both monogenean species attached to the host fish, although the viability of the eggs was low; the treatment was unable to prevent viable eggs from hatching as well. Both formalin treatments (250ppm and 400ppm) were effective at removing the ectoparasites. The 400ppm treatment was more effective at eliminating *Z. seriolae* (99% removed as opposed to 49% for the 250ppm treatment), while both treatments removed about 80% of the attached *B. seriolae*. Any remaining *B. seriolae* failed to produce eggs, while the remaining *Z. seriolae* still laid eggs but had a very low hatch rate (Sharp et al., 2004).

Another current treatment that is used is freshwater baths, however logistically it is not practical in sea cage farms (Sharp et al., 2004; Ernst et al., 2005; Sepúlveda and Gonzalez, 2014). The ectoparasites require saline water, therefore they can't survive full immersion in fresh water (salinity below 15ppt). At this salinity the eggs also take a very long time to hatch or don't hatch at all. This treatment is short-term, however, because the eggs and parasites in the water are not treated and can re-infect treated fish in as little as 24 hours (Ernst et al., 2005). Garlic *(Allium sativum)* extract has also been tested for efficacy due to the antibiotic effects of the allicin in the herb. Allicin concentrations of

15.2 μL L^{-1} were effective at minimizing hatch success and oncomiracidial longevity of *Neobenedenia* sp. in barramundi (*Lates calcarifer*). Lower concentrations (0.76 and 1.52 μL L^{-1} were effective to a lesser extent as well (Militz et al., 2013a).

B. seriolae eggs are sensitive to heat and desiccation as well, therefore exposure to hot tap water at 50°C or higher for 30 seconds would kill any parasites, however this is not a practical solution. Leaving the tank completely dry for as little as 3 minutes can result in effective sterilization as well (Ernst et al., 2005). This is one primary benefit of flippable sea-spar sea cages, however parasites attached to the fish would require further treatment. Immersion in 25% ethanol, while more expensive, will also kill the eggs; sodium hypochlorite at concentrations up to 1000ppm, however, had little to no effect (Ernst et al., 2005). Exposure to in-tank water temperatures of over 30°C for more than 48 hours was shown to kill about 98% of *B. seriolae* eggs. This is even more efficient if coordinated with chemical treatment to prevent re-infection. These elevated temperatures increase the chances of lower dissolved oxygen (DO) levels and may increase stress so bath treatments aren't recommended. While not feasible for sea cages, one potential biological control of monogenean parasites is the use of cleaner gobies, specifically the cleaning goby *Elacatinus genie* and the neon goby *Gobiosoma oceanops*, which have been observed to control parasitic populations in Florida red tilapia production (Whittington and Chisholm, 2008). These alternative bath treatments have been trialed and used in some situations, primarily in labs or indoor locations, as they are ill suited for treatment in sea cage scenarios.

1.3.2 In-feed Treatments

Oral administration is favorable to bath treatment for several reasons. Giving baths, especially in sea cage culture, is labor intensive, weather dependent, time consuming, and also stressful to the fish themselves, possibly making them more susceptible to further infection. It is also difficult to successfully calculate dosage rates, especially with the more toxic treatment chemicals, and some mortality still occurs (Williams et al., 2008). Treatments incorporated into the feed regimen require much less labor and time and minimizes stress in the fish as well. This practice is also more cost-effective because there is less infrastructure required, fewer hours worked, and potentially less of the treatment chemical involved (Williams et al., 2008).

Kim and Choi (1998) used in-feed mebendazole and bithionol (1.25g/kg) to treat monogenean infections (*Microcotyle sebastis*) in rockfish (*Sebastes schlegeli*) and observed significantly fewer parasites than the control group. Other chemicals that have been trialed as in-feed medications include caprylic acid, orange oil, peppermint oil, and cinnamon oil, of which only caprylic acid successfully prevented horizontal monogenean infection in the tiger puffer (*Takifugu rubripes*) (Hirazawa et al., 2000). Febendazole (2.5g/kg) has been used to treat monogenean infections in silver perch (*Bidyanus bidyanus*), exhibiting 95% efficacy (Forwood et al., 2013).

Garlic (*Allium sativum*) has been tested as dietary supplement due to the antibiotic capacity of allicin, one of the active ingredients. Garlic inclusion reduced *Neobenedenia* sp. infections in barramundi by up to 70% after 30 days, however minimal effects were recorded after only 10 days, suggesting a delayed response by the fish (Militz et al., 2013b).

This emphasizes the need for in-feed treatments to be used in coordination with water quality control (Ernst, 2005). Praziquantel has the most potential of any in-feed compound due to its availability and proven efficacy.

1.3.3 Praziquantel

Praziquantel (PZQ), also known as EMBAY 8440, is comprised of a central pyrazino isoquinoline ring compound that lends the drug its antiparasitic capacity (Donato and Pica-Mattoccia, 2003). These effects were first observed in the early 1970's, and the "anticestode and antitrematode" impacts on animals and humans were published in 1977 and 1978, respectively (Thomas and Goennert, 1977; Goennert and Andrews, 1977; Leopold et al., 1978). The drug was described as a "nearly white crystalline powder of bitter taste" and was initially used primarily to treat schistomiasis and cestode infections in humans and causes minimal side effects (Donato and Pica-Mattoccia, 2003; Thoney, 1990). It is also effective in treating hydatid disease, caused by canine tapeworms, and prevents the disease's spread to humans and livestock (Liang et al., 2009). Praziquantel causes contraction and "spastic paralysis" in parasites by increasing muscle contraction; the drug damages the parasite's tegument as well (Maartens and Sinxadi, 2009).

Due to its antihelmintic properties, PZQ has since been expanded to aquatic treatment. The drug has been used to treat the polyopisthocotylean monogeneans *Microtyle sebastis* in the rockfish *Sebastes schlegeli* (Kim and Cho, 2000), *Heterobothrium okamatoi* in the tiger puffer *Takifugu rubripes* (Hirazawa, 2000), and *Sparicotlye chysophrii* in gilthead sea bream *Sparus aurata* L. (Sitjà-Bobadilla, 2006) as

well as the monopisthocotylean monogeneans *Gyrodactylus* spp. of rainbow trout *Oncorhynchus mykiss* (Tojo and Santamarina, 1998) and *Neobenedenia girellae* in the spotted halibut *Verasper variegates* (Hirazawa et al., 2004).

1.3.3.1 Praziquantel Bath Treatment

Sharp et al. (2004) showed that 2.5ppm praziquantel bath treatments for 24 (P24) or 48 (P48) hours removed about 99% of *B. seriolae* and *Z. seriolae* from yellowtail kingfish *Seriola lalandi lalandi*. Further examination of the ectoparasites revealed vacuolization of the tegument. After the P24 treatment, one remaining *B. seriolae* laid eggs, whereas after the P48 treatment there were no parasites left on the host fish. All of the eggs laid, however, were viable. There were no *Z. seriolae* remaining after P24, and only one after P48; the one remaining individual successfully laid eggs but they never hatched. A separate treatment on just the eggs of both monogenean species showed that neither P24 nor P48 could significantly reduce viability (>66%). PZQ bath treatments should therefore last for longer than 24 hours because even though only one *B. seriolae* individual remained, it successfully laid viable eggs (Sharp et al., 2004). Studies by Schmahl and Taraschewski (1987) (as cited in Sharp et al., 2004) and Mitchell (1995) support this theory, as they found that vacuolization and efficacy against other monogeneans (*Gyrodactylus aculeate* and digenetic trematodes) increased with time as opposed to concentration. Longer treatments also allow for the use of lower concentrations, reducing the total treatment cost. The praziquantel treatments (P24 and P48) used in the study by Sharp et al. (2004) were not significantly different than the 200ppm and 400ppm formalin treatments. All of the above treatments, however, were

very effective in simulated conditions with very high infection rates. Proper management, including quarantine and disease management should prevent such radical infection rates in hatcheries and farms, potentially making these treatments even more potent. A secondary bath treatment is also recommended, for while the first treatment will eliminate the adult worms, the second treatment will disrupt the life cycle by killing any immature ectoparasites before they have the chance to reinfect the fish (Sharp et al., 2004). PZQ has been measured to stay in the plasma and muscle tissue for 72 and 24 hours, respectively. This may serve as a guide for how long to wait between treatments (Kim et al., 2001a).

Noga (2010) compiled PZQ bath treatment protocols in the book *Fish Disease Diagnosis and Treatment*. According to this compilation, the recommended bath concentration for monogeneans is 20mg/L in a bath for 1.5 hours. Juvenile fish may be sensitive to these higher doses, however a concentration of 10mg/L for 3 hours is more tolerable and also more cost effective. Similar concentrations and durations have also been used to treat *Diplostomum spathaceum* in carp (S*zékely and Moln*ár, 1991 as cited in Noga, 2010). The efficacy of PZQ bath treatments is more effective when it is dissolved in dimethyl sulfoxide (DMSO) as opposed to ethanol (Noga, 2010).

The disadvantages of praziquantel bath treatments include logistical difficulty, the use of reagents for dissolving the treatment, and the increased stress on the fish. PZQ baths exhibit limited feasibility in sea cage environments as well due to the same issues with any bath treatment, while in-feed incorporation streamlines the process. Bath treatments are also economically inefficient compared to in-feed methods because a larger amount of the medication is required.

1.3.3.2 Praziquantel Oral Treatment

Oral administration of PZQ to treat monogenean infections may involve mixing the drug directly with the feed. Feeding 20g of PZQ per kg of feed at 1% of the total fish bodyweight every other day for a total of 3 feeding days is effective for treating *Microcotyle sebastis* parasites (Kim and Cho, 2000). Administering 100mg of PZQ per kg of body weight by direct intubation of the stomach has been shown to severely reduce the number of *B. seriolae* and eliminate *Z. seriolae* infection in *Seriola lalandi*, however this trial served to demonstrate the efficacy of oral administration, not to serve as a treatment option. The total treatment should be split in to 4 doses daily and treat the fish every third day (Williams et al., 2008). PZQ was also very effective as an in-feed treatment against *B. seriolae* at 150mg/kg body weight for *Seriola dumerili* and *Seriola quinqueradiata*, however it required higher concentrations to treat *Neobenedenia girellae* parasites (Hirazawa et al., 2013). Other oral treatment techniques with PZQ have been successfully used for carp (S*zékely and Moln*ár, 1991 as cited in Noga, 2010), trout (Bylund and Sumari, 1981) and chub mackerel (*Scomber japonicus*) (Yamamoto et al., 2011).

Partridge et al. (2014) demonstrated high feed intake levels in large yellowtail kingfish (3.5kg average). PZQ microcapsules included in the mash exhibited 84±8% intake while PZQ powder included in the mash resulted in 90±6% consumption. Surface coated PZQ powder, however, resulted in 79±2% consumption, significantly lower than surface coated microspheres (102±3%). The surface coated diets were not significantly different from PZQ incorporated into the mash. Partridge et al. (2014) demonstrated that surface coated powder and microcapsules exhibited lower palatability at higher PZQ

inclusion (77±9% at 16g/kg and 19±5% at 25g.kg for microcapsules and 9±9% for PZQ powder at 16g/kg).

Other drugs administered via oral treatments are mebendazole (Kim et al., 1998) and PZQ in combination with cimetidine, which suppresses the metabolism of PZQ and therefore increases bioavailability in the blood to affect more parasitic individuals. This could also potentially lower the total dosage of PZQ required, and accordingly, the overall cost (Kim et al., 2001b). PZQ has been measured to remain in the plasma and muscle tissue for 96 hours each, indicating the full length of the treatment time; further feeding, however, will augment the PZQ concentration in the blood (Kim et al., 2001b).

1.3.3.3 Palatability Issues

One issue with the oral administration of PZQ is that the drug is very bitter and fish will reject the medicated feed (Williams et al., 2008). This could explain why the lower concentration of PZQ (50 and 75mg kg^{-1} BW d^{-1} of feed as opposed to 100 and 150) was more effective in treating *B. seriolae* and *Z. seriolae* in yellowtail kingfish. Williams et al. (2008) highlight palatability as the central issue preventing PZQ from becoming useful in feed treatment for monogenean parasites despite their attempts to use fish oil to mask the bitter flavor. PZQ palatability issues have also been noted in *Seriola quinqueradiata* and *Seriola dumerili* in Japan, have been observed to reduce the appetite of the spotted halibut *Verasper variegates* at 150 mg kg^{-1} BW d^{-1} (Hirazawa et al., 2004), and are problematic for the gilthead sea bream *Sparus auratus* **(**Sitjà-Bobadilla, 2006). Palatability issues are also typically more problematic in large fish as opposed to small fish. This is because large fish eat a smaller percentage of their body weight and therefore

require a higher PZQ dietary inclusion level, the primary factor contributing to the palatability issue (Partridge et al., 2012).

When the fish reject pellets it can make it difficult to determine whether the required dose was administered. Pellet rejection also negatively impacts the economics of a commercial aquaculture operation: the medication is expensive, meaning that uneaten pellets are wasteful and prolonged feeding of medicated feed can result in slower growth rates over time and less profit. Other masking alternatives such as microencapsulation or starving prior to feeding the medicated diet are undesirable as well because of either the expense or the slowed growth, respectively (Williams et al., 2008).

Some trials have overcome palatability issues by incorporating the praziquantel into the feed during the extrusion process (Hirazawa et al., 2004; Kim et al., 2003). This process, however, could potentially destroy the medication via pressure, high temperature or humidity (Vertommen and Kinget, 1998 as cited in Williams et al. 2008). Partridge et al. (2012) incorporated praziquantel into the feed for juvenile *Seriola lalandi* in Australia, however this did not seem to alleviate any palatability issues and further masking was required. Furthermore, surface-coating pellets is sensible for farm settings because the process is simple. Adding PZQ to the mash is more complicated and minimizes flexibility of dose rates (Partridge et al., 2012). Gelatin and casein have been trialed as coating agents as well, augmenting palatability at 2.5g/kg (allowing for a daily dose of 100mg/kg/day in 150g fish and 65g/kg/day in 500g fish), however at higher concentrations, such as 10g/kg of pure PZQ, feed rejection was observed due to palatability issues (Partridge et al., 2012). Fish hydrolysate, however, may help to reduce the smell of PZQ in the feed. Another potential solution to the palatability concerns with

praziquantel is to incorporate the drug into microspheres within the feed. Trials that included PZQ at up to 4.6g/kg demonstrated no effect on palatability for juvenile yellowtail kingfish (Partridge et al., 2012). In an efficacy trial with approximately 3.95g yellowtail kingfish, pellets with 16g/kg of enteric microcapsules or surface-coated pure PZQ were compared. Both pellets were also surface coated with aniseed and gelatin. The enteric PZQ pellet resulted in some feed rejection (approximately 75% intake), while the surface-coated PZQ had nearly complete rejection, and subsequently exhibited poor parasite treatment (Partridge et al., 2012). This trial determined that 2.5 grams of PZQ/kg is sufficient for yellowtail kingfish (*Seriola lalandi*) under 500g, while larger fish could potentially receive enteric microcapsules with concentrations of 16g/kg in order to deliver enough medication. The medication should be delivered for a minimum of seven consecutive days, though it may be shorter if only treating gill flukes (Partridge et al., 2012). While there is a dietary PZQ treatment developed for *Benedenia seriolae* parasitizing *Seriola quinqueradiata* in Japan (Hadaclean® Bayer) despite palatability issues, palatability is still a problem with *Seriola lalandi* in Australia.

1.4 Purpose

This trial was designed to investigate palatability issues of praziquantel as a dietary treatment for juvenile yellowtail kingfish *Seriola lalandi*. The purpose was to use different concentrations of garlic extract (GB1) to surface-coat a feed previously extruded with praziquantel to see if the garlic flavor could overcome the bitter characteristics of drug. Garlic was selected as a masking agent because of its strong flavor and because it has been used before by Militz et al. (2013b) as a dietary inclusion with no recorded

negative palatability effects. Garlic also exhibits its own antibiotic capabilites, a trait which could then be further trialed in the future. The ability to use garlic in a commercial setting would allow for adequate dosage rates to maximize treatment capability without significant feed rejection. This would minimize the decline in growth rate during the treatment period and facilitate the deliverance of accurate doses of PZQ in pellet feeds. The ability to deliver a known amount of feed is important if the PZQ is included in the pellet prior to extrusion because the amount of PZQ/kg of feed cannot be altered without adding an additional surface coating. High feed intake percentages mean that the fish are receiving the approximate dose required without having to measure intake to compensate for uneaten or rejected pellets.

Chapter 2: Materials and Methods

The trial was completed at the Australian Centre for Applied Aquaculture Research (ACAAR) in Fremantle, Australia (1 Fleet St., Fremantle WA, Australia). The facilities and all necessary materials and chemicals were generously offered by ACAAR and their staff to provide for the trial and ensure its completion.

2.1 Setup

The trial took place in an isolated room to separate the test subjects from the other fish located throughout the hatchery for the purpose of biosecurity. Two x 3,500 liter rectangular baths were placed parallel to each other and each held 10 x 180 liter blue barrels (20 total). The baths served to collect waste drainage from the barrels and allow the effluent water to flow out of the room. The water intake came from the main ACAAR intake line, originating at a well drilled into the sand and naturally filtered to <5μm. Both of the 10-barrel arrays received water from separate intake lines, which further branched off to each individual barrel. The intake lines fed into a 1-inch PVC pipe attached to the inside rim of the barrel and set at an angle to create a gentle vortex. This vortex concentrated waste matter in the center of the barrel, where another 1-inch PVC pipe served as a drainage outlet, effectively creating a self-cleaning setup within each barrel. The approximate water exchange through the barrels was once every two hours. Each barrel was further outfitted with one fine-pore ceramic aeration stone that served to mix and aerate the water and covered with a loose mesh netting to prevent fish from jumping out.

The trial room was lighted using halogen lights attached to a timer to allow for total light hour regulation. The lights would be manually turned on at 8:15-8:30 AM and would be set to turn off at 4:00 PM for a total daylight hour time of 7:30-7:45 hours. The 3,500 liter baths and 180 liter barrels were all cleaned using a diluted oxalic acid solution and rinsed thoroughly prior to filling the system with water and stocking.

2.2 Stocking

Each tank was stocked with juvenile yellowtail kingfish (*Seriola lalandi*) from a stock already existing in an ACAAR holding tank. The fish were manually netted out of the tank and into a separate container with a low dose of Aqui-S anesthetic (20ppm) to minimize stress while handling and weighing each individual. After weighing each fish, it would be placed into a bucket labeled 1-10 and corresponding to a specific barrel in the trial room. Each bucket, and therefore barrel, received a total of 5 fish. Stocking 10 barrels at once using buckets allowed for the average fish weight to be compared and balanced between all of them. Once completed, this process was then repeated for the second 10 barrels, using the average weight from the first 10 as a guide for stocking.

The average weight of all 100 fish in the trial was 95.2±1.3 g with a range of 70-123g. The following table (**Table 1)** shows each individual barrel and its initial stocking record.

2.3 Acclimation Feed

The test fish were fed an acclimation diet (Skretting's commercial 3mm pellets) for 5 days prior to the test diet. This allowed them to acclimate to the barrel environment as well as the pellet size. The diet was stored in a walk-in cool room at approximately

6°C and weighed prior to feeding to measure consumption. The initial amount fed was determined according to **Table 2**, which shows predicted consumption by %BW/day.

Table 1 shows the weights of the fish stocked in each of the barrels. The average weight, standard error, and total weight are shown for each barrel as well.

	1	2	3	4	5	6	7	8	9	10
1	95	120	99	102	88	98	105	95	82	96
2	110	112	104	91	97	110	93	84	91	87
3	105	80	90	94	101	90	94	103	103	101
4	89	75	83	111	108	92	92	98	101	109
5	77	97	105	70	76	84	101	103	102	86
Average	95.2	96.8	96.2	93.6	94.0	94.8	97.0	96.6	95.8	95.8
SE	5.9	8.7	4.2	6.8	5.5	4.4	2.5	3.5	4.1	4.3
Total	476	484	481	468	470	474	485	483	479	479
	11	12	13	14	15	16	17	18	19	20
1	72	79	80	71	93	74	73	74	76	71
2	99	78	96	83	94	117	111	120	97	118
3	106	95	92	112	100	88	96	81	123	85
4	95	111	101	96	112	85	87	120	78	122
5	103	107	104	106	77	117	101	85	96	88
Average	95.0	94.0	94.6	93.6	95.2	96.2	93.6	96.0	94.0	96.8
SE	6.0	6.9	4.2	7.5	5.7	8.8	6.4	10.0	8.5	9.9
Total	475	470	473	468	476	481	468	480	470	484

Since the average fish weighed 95.2 ±1.3g, the feed amount was rounded up to the 100g row on the table. The ambient water temperature was 22°C, therefore each fish should receive approximately 4.3% of its total body weight daily, multiplied by 5 because there were 5 fish in each barrel. The total daily feed required during the acclimation period was 21.4g/day split into two feedings, a morning feed at 9:30AM and an afternoon feed at 2:30PM, each with 10.7g per barrel.

Table 2 shows the feed amount that kingfish need in %BW/day. The leftmost line shows body weight and topmost shows water temperature.

		Temperature (°C)														
		14	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
Weight of Fish (kg)	**10**	6.3	6.8	7.3	7.7	8.2	8.6	9.0	9.4	9.8	10.1	10.5	10.8	11.2	11.5	11.8
	20	5.7	6.2	6.6	7.0	7.5	7.8	8.2	8.6	8.9	9.3	9.6	9.9	10.2	10.5	10.8
	30	4.7	5.1	5.5	5.8	6.2	6.5	6.8	7.1	7.4	7.7	7.9	8.1	8.4	8.7	8.9
	40	4.1	4.4	4.8	5.1	5.4	5.6	5.9	6.2	6.4	6.7	6.9	7.1	7.3	7.5	7.8
	50	3.7	4.0	4.3	4.6	4.8	5.1	5.3	5.5	5.8	6.0	6.2	6.4	6.6	6.8	7.0
	100	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.1	4.3	4.5	4.6	4.8	4.9	5.1	5.2
	200	2.2	2.3	2.5	2.7	2.8	3.0	3.1	3.2	3.4	3.5	3.6	3.7	3.8	4.0	4.1
	300	1.9	2.0	2.2	2.3	2.4	2.6	2.7	2.8	2.9	3.0	3.1	3.3	3.4	3.4	3.5
	400	1.7	1.8	2.0	2.1	2.2	2.3	2.4	2.5	2.7	2.8	2.8	2.9	3.0	3.1	3.2
	500	1.6	1.7	1.8	1.9	2.0	2.2	2.3	2.4	2.5	2.5	2.6	2.7	2.8	2.9	3.0
	700	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.2	2.3	2.4	2.5	2.5	2.6
	1000	1.2	1.3	1.4	1.5	1.6	1.7	1.7	1.8	1.9	2.0	2.0	2.1	2.2	2.2	2.3
	1250	1.1	1.2	1.3	1.4	1.4	1.5	1.6	1.7	1.7	1.8	1.9	1.9	2.0	2.0	2.1
	1500	1.0	1.1	1.2	1.3	1.3	1.4	1.5	1.5	1.6	1.7	1.7	1.8	1.8	1.9	1.9
	1750	1.0	1.0	1.1	1.2	1.3	1.3	1.4	1.4	1.5	1.6	1.6	1.7	1.7	1.8	1.8
	2000	0.9	1.0	0.1	1.1	1.2	1.3	1.3	1.4	1.4	1.5	1.5	1.6	1.6	1.7	1.7
	2500	0.8	0.9	1.0	1.0	1.1	1.1	1.2	1.3	1.3	1.4	1.4	1.5	1.5	1.5	1.6
	3000	0.8	0.8	0.9	1.0	1.0	1.1	1.1	1.2	1.2	1.3	1.3	1.4	1.4	1.4	1.5
	3500	0.7	0.8	0.9	0.9	1.0	1.0	1.1	1.1	1.2	1.2	1.2	1.3	1.3	1.4	1.4
	4000	0.7	0.8	0.8	0.9	0.9	1.0	1.0	1.1	1.1	1.1	1.2	1.2	1.3	1.3	1.3
	4500	0.7	0.7	0.8	0.8	0.9	0.9	1.0	1.0	1.1	1.1	1.1	1.2	1.2	1.2	1.3
	5000	0.7	0.7	0.8	0.8	0.9	0.9	0.9	1.0	1.0	1.1	1.1	1.1	1.2	1.2	1.2
	5500	0.6	0.7	0.7	0.8	0.8	0.9	0.9	1.0	1.0	1.0	1.1	1.1	1.1	1.2	1.2
	6000	0.6	0.7	0.7	0.8	0.8	0.9	0.9	0.9	1.0	1.0	1.1	1.1	1.1	1.2	1.2

The feeds were weighed to within 0.02g of the target weight using an electronic balance and sorted into small plastic vials labeled with their corresponding barrel number to ensure that the same vial was used each time. The exact weight of the feed pellets was recorded. 100 pellets were hand-counted and weighed separately to determine an average of 0.03g/pellet. Each plastic vial was weighed empty as well and recorded on a spreadsheet. Immediately prior to feeding, the aeration was turned off to allow the pellets to remain in the water column for as long as possible, as the fish rarely ate pellets that settled to the bottom of the tank. The pellets were slowly dropped into the tank under careful observation to see how the fish were eating. The feed period was 3:00 minutes to standardize the variable for all barrels. After the 3:00 time period, all remaining uneaten pellets in the tank were counted and recorded on a spreadsheet. This process was repeated for all 20 barrels.

After feeding, the total weight of the vial and remaining feed was measured and recorded. The spreadsheet formula would then automatically deduct the empty vial weight from this total. The total number of uneaten pellets was recorded as well and multiplied by 0.03 to determine their total weight. This number was then used to measure the total feed consumed and the percent consumption. After weighing the vials post-feed, they would be refilled to the target weight (10.7g) for the next feed session. The acclimation feed was fed and recorded for 3 days.

2.4 Trial Feed

The trial feed in the study was manufactured by the nutrition department of The Commonwealth Scientific and Industrial Research Organization (CSIRO), Australia's national science agency. The praziquantel, obtained from a local veterinary clinic, was mixed into the mash at 5g/kg prior to the feed extrusion process for the trial feed. The control diet was Skretting's commercial 3mm pellet, the same as the acclimation feed. The masking agent tested to cover the bitter flavor of PZQ was garlic (*Alluvium sativum*). The garlic used was a liquid concentrate to facilitate surface coating the pellets, from here on referred to as GB1. **Figure 1** shows the layout of the 20 barrels and the feed regimen that each received. The numbers were selected randomly for each treatment.

The trial feed was made by surface coating the 3mm pellets with the GB1 at different concentrations. The GB1 was measured in a graduated cylinder and then poured over a known amount of feed in a mixing bowl. The GB1 was manually mixed in for 10 minutes to ensure that all of the pellets were sufficiently coated. The feed was then placed into flat trays and spread evenly before placing it into the walk-in cool room at 6°C to dry overnight before being fed. All trial feed was mixed and dried prior to the first day of feeding. The five feed regimens are summarized in **Table 3** and were: a control diet (Skretting 3mm commercial pellet), the control diet mixed with 50ml GB1/kg of feed, a pure PZQ pellet, the PZQ diet with 20ml GB1/kg and the PZQ diet with 50ml GB1/kg. The control diet with GB1 was fed to determine whether, in the case of feed rejection, the garlic was the source of the issue.

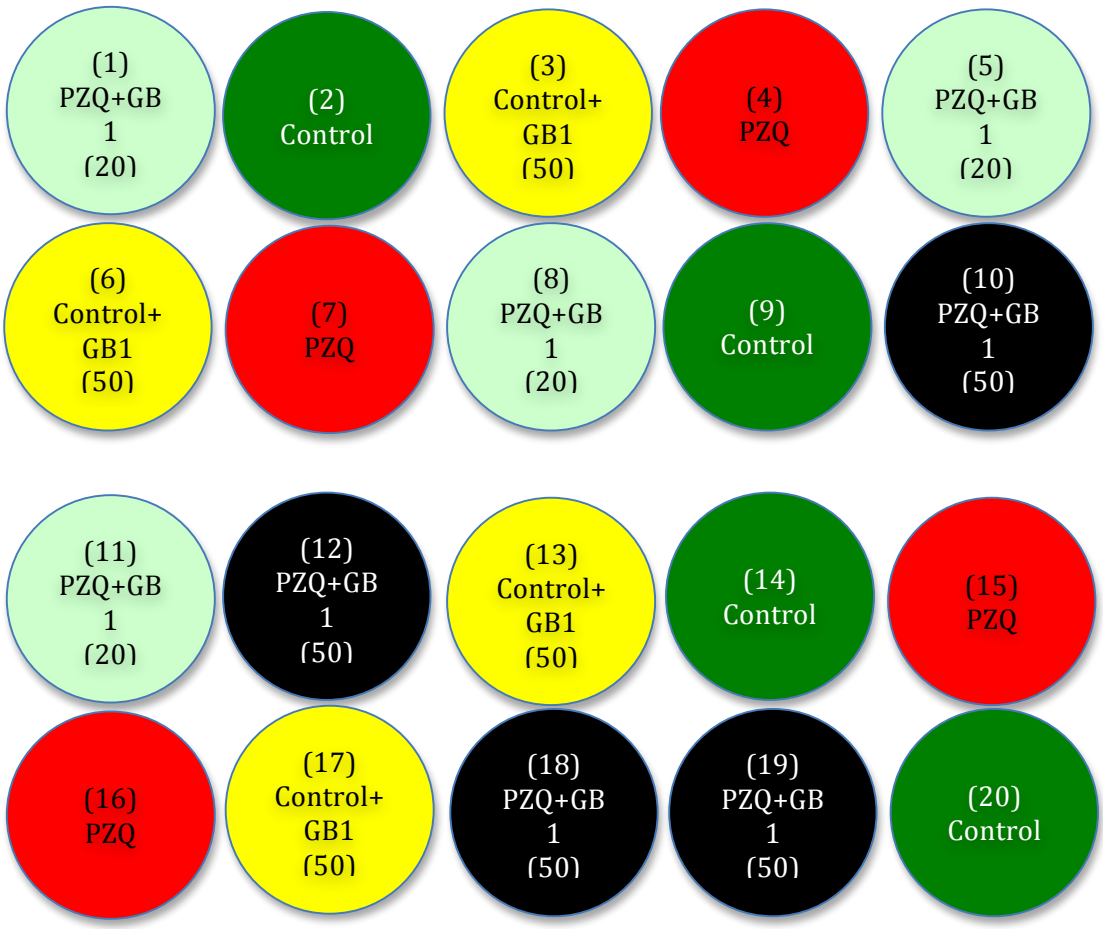

Figure 1 shows the 20 treatment barrels and the feed regimen that they received. Control + GB1(50) is the control diet surface coated with 50ml of GB1/kg, PZQ represents the pure praziquantel feed, PZQ+GB1(20) is the PZQ diet surface coated with 20ml/kg GB1 and PZQ+GB1(50) has 50ml GB1 per kg of feed.

The trial feed was delivered twice a day for 4 days following the exact same routine as the acclimation feed. The same plastic vials were used to measure the feed, and each vial was wrapped in a different color tape corresponding to the treatment for that barrel (See **Table 2**). 4 barrels were designated for each feed regiment to ensure sufficient replication for statistical significance. The morning feed on the fifth day returned to a control diet for all 20 tanks to observe recovery after the trial diets. The

acclimation feed trials revealed that fish consumed 60% of the ration recommended in **Table 3**. The ration fed during the trial was therefore adjusted to 5g/feed (±0.02g) (2.1%BW/day) in order to ensure that the fish receiving the control diet could achieve 100% consumption.

2.5 Statistical Analysis

A one-way ANOVA was performed on the average daily intake results to determine if there was a significant difference (±95% confidence interval). The operation was performed using the JMP analytical computer software program. The total average consumption values were standardized using the arcsine value for each prior to testing the data. A repeated measures ANOVA was performed as well to determine whether there was a significant change in the data over time, and a final one-way ANOVA was performed on the recovery feed consumption data to determine whether there was a significant difference.

Table 3 illustrates the different treatments and their corresponding vial colors to facilitate differentiation.

Treatment Color	Treatment
	Control
	Control + 50ml/kg GB1 (GB1(50))
	PZQ at 5g/kg
	PZQ at 5g/kg + 20ml/kg of GB1 (PZQ + GB1(20))
	PZQ at 5g/kg + 50ml/kg of GB1 (PZQ + GB1(50))

Chapter 3: Results

3.1 Acclimation Feed

The overall consumption average was 59.38%, with an average of 57.61% for the AM feed and 61.15% for the PM feed. Therefore, the trial diet was set at 5g/feed for a total feed ration of 10g/day as opposed to the 10.7g (21.4g total) feedings during the acclimation period. While 5g is only 46.72% of 10.7g (as opposed to 59.38%), the percentage represents the average consumption and a smaller number is required to guarantee nearly 100% consumption of the trial feed in the control group, setting a clear baseline with which to compare the other experimental feeds. The acclimation feed percentages were only measured from Wednesday through Friday, with the Saturday and Sunday feeds measured out prior to feeding but no follow-up measurements. During the 3-day period for which both AM and PM measurements were taken, the fish ate nearly evenly between the 2 feeds overall, with 48% of the daily consumption during the AM feed and 52% during the PM feed.

3.2 Trial Feed

The trial results indicate a significant effect of diet type on the feed palatability ($P<0.0001$) (see **Figure 5**). The fish receiving the PZQ + GB1(50) diet displayed a consumption rate (88±10%) equivalent to the control (97±2%) and control + GB1(50) (99±0%) diets. There was no significant difference between these three diets. The PZQ diet consumption (44±8%) was equal to the PZQ + GB1(20) (47±5%); these diets exhibited significantly different consumption rates than the first three. The latter diets,

however, were not significantly different from each other. **Figure 4** shows the total feed amounts and total average consumption for each of the trial barrels during the study.

The repeated measures ANOVA confirms that there is no significant change in the total average feed intake over time for each individual treatment: control ($p=0.45$), PZQ + GB1(20) ($p=0.32$), PZQ ($p=0.87$), control + GB1(50) ($p=0.36$) and PZQ + GB1(50) ($p=0.38$).

The one-way ANOVA analysis regarding the final recovery feed indicated no significant difference between any of the trial diets ($p=0.86$).

Table 4 shows the total amount of feed given to each treatment barrel (not including the recovery diet) and further depicts the total average consumption for each replicate.

Barrel Number	Treatment	Total AM Feed	Total PM Feed	Total Feed	Total Average Consumption
1	PZQ + GB1 (20)	19.99	19.98	39.97	34%
2	Control	19.99	20.00	39.99	100%
3	Control + GB1 (50)	19.97	20.01	39.98	99%
4	PZQ	19.99	20.00	39.99	38%
5	PZQ + GB1 (20)	19.99	19.97	39.96	59%
6	Control + GB1 (50)	20.00	20.01	40.01	99%
7	PZQ	20.04	20.01	40.05	61%
8	PZQ + GB1 (20)	19.99	20.01	40.00	52%
9	Control	19.98	19.98	39.96	91%
10	PZQ + GB1 (50)	20.00	20.01	40.01	100%
11	PZQ + GB1 (20)	19.98	20.00	39.98	43%
12	PZQ + GB1 (50)	19.98	20.00	39.98	96%
13	Control + GB1 (50)	20.00	20.02	40.02	99%
14	Control	20.03	20.00	40.03	99%
15	PZQ	19.97	20.00	39.97	50%
16	PZQ	20.01	20.00	40.01	26%
17	Control + GB1 (50)	20.01	20.01	40.02	99%
18	PZQ + GB1 (50)	20.00	19.97	39.97	57%
19	PZQ + GB1 (50)	20.02	20.03	40.05	98%
20	Control	20.01	19.99	40.00	100%

Table 5 shows the daily and overall average intakes for each of the treatment diets. It shows the standard error for each average calculation as well.

Daily Average	Monday	Tuesday	Wednesday	Thursday	Friday recovery	Overall Average	
Control	97±2%	97±2%	98±1%	97±3%	98±2%	Control	97±2%
PZQ + GB1 (20)	45±5%	54±6%	42±4%	46±9%	98±1%	PZQ + GB1 (20)	47±5%
PZQ	43±8%	47±9%	42±10%	44±6%	98±1%	PZQ	44±8%
Control + GB1 (50)	100±0%	99±0%	99±1%	99±0%	100±0%	Control + GB1 (50)	99±0%
PZQ + GB1 (50)	88±77%	91±9%	83±16%	90±10%	97±3%	PZQ + GB1 (50)	88±10 %

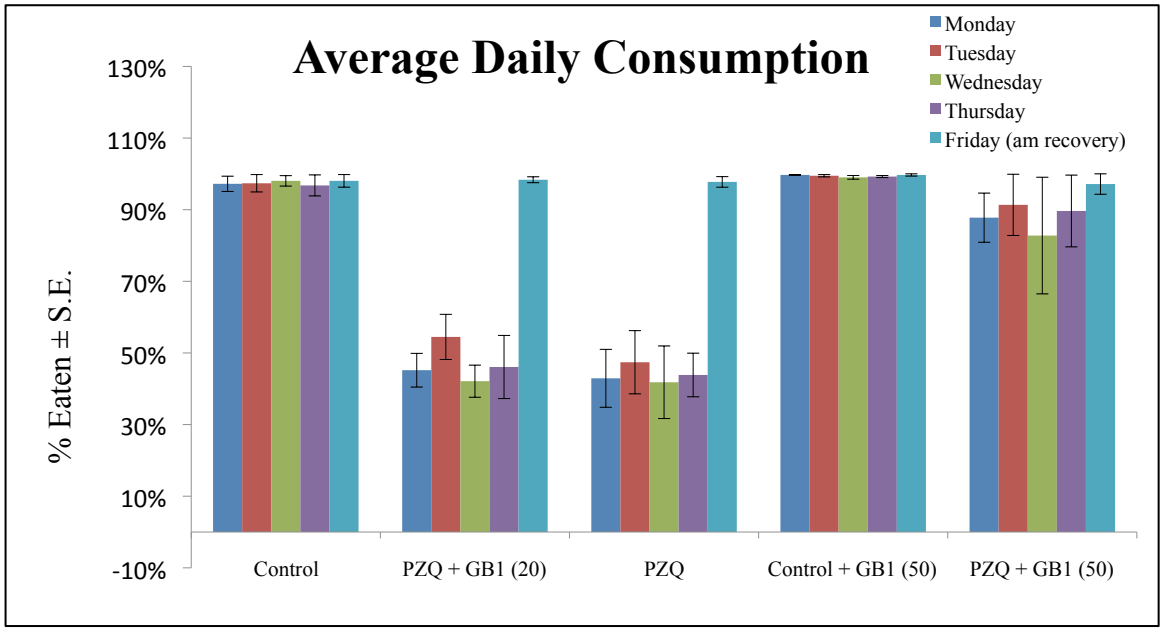

Figure 2 shows the average daily consumption for each treatment, including standard error bars.

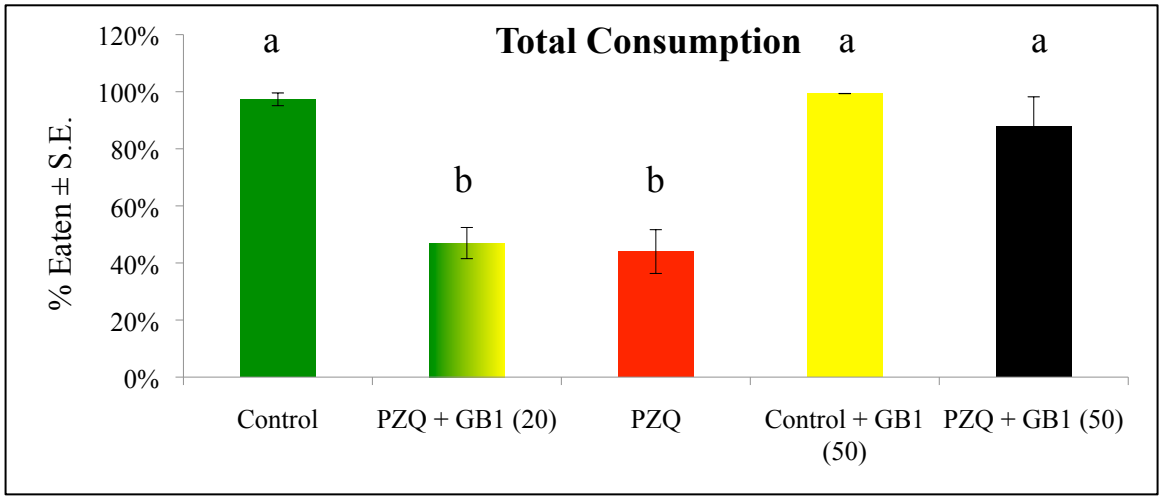

Figure 3 shows the total consumption for each treatment diet and the associated standard error bars. The letters above each data point represent their respective statistical group according to the JMP one-way ANOVA test.

Chapter 4: Discussion

4.1 GB1 Analysis:

This trial shows that 50ml of GB1 surface-coated onto 3mm pellets with PZQ incorporated into the mash (5g/kg) is sufficient to mask the bitter flavor of the medication and to significantly increase the overall palatability of the feed ($p<0.0001$). GB1 surface-coated at 20ml/kg and pure PZQ feeds, however, caused high rejection levels (<50% consumption), rendering the medication essentially useless due to dosage and economic concerns. The control feed surface-coated with 50ml/kg of GB1 was a safeguard to ensure that, should all three PZQ feeds show high rejection levels, the garlic was not the cause of rejection. This can be confirmed due to the results observed.

One concern was that the average consumption levels for each treatment might decrease over time for the duration of the trial. The repeated measures ANOVA confirmed that there was no significant fluctuation in consumption throughout the study ($p>0.3$ for all treatments) and that the intake was consistent for each trial feed. The recovery feed (control diet) fed the final morning showed no significant difference between any of the treatment barrels as well ($p=0.86$). This suggests that the fish are sensitive to the smell/taste of the praziquantel medication, and that PZQ was the specific reason for reduced consumption in the PZQ and PZQ + GB1(20) treatments.

Variability among the trial barrels caused daily standard error percentages as high as 16% during the trial period (in the PZQ+GB1(50) treatment) and an overall maximum of 10% for the entire trial period. Variability could be attributed to the fact that there were only 5 fish in each tank, therefore if one of the fish was not eating a normal ration

the results would be affected. However, the control diets ate well with little standard error, as expected after the feed amount was adjusted according to the acclimation results.

While Partridge et al. (2012), using *S. lalandi*, first attempted to include PZQ into the feed mash prior to the extrusion process, there were still significant palatability issues observed. Garlic paste, however, at a concentration of 50g/kg of feed, has proven to be a suitable masking agent to greatly reduce palatability issues (88±10% consumption). GB1 masking increases palatability more than microcapsule incorporation as well, as the microcapsules only resulted in approximately 75% consumption during an efficacy trial, however the enteric capsules contained a higher concentration of PZQ (4.6g/kg) (Partridge et al., 2012). Partridge et al. (2014) also found that PZQ powder incorporated into the mash at 8g/kg resulted in 90±6% consumption and that enteric microcapsules resulted in 84±8% consumption, however these results were observed in 3.5kg fish with no set time limit to measure feed intake. These results could be supplemented by testing the same diets with garlic as a masking agent. Dose rates were also significantly less than the 105mg/kg/day administered in this trial (53.7mg/kg/day for the microcapsules in the mash).

4.2 Limitations:

This trial was solely to determine the effects of GB1 on palatability, and no efficacy trials were undertaken to determine the potential effects of the masking agent due to the fact that fluke infected fish were not available. During the trial period, the fish received a daily amount of 50mg PZQ spread over the two feeds (10g feed/day at 5g PZQ/kg of feed), meaning that each fish received 10mg of PZQ. This results in a total

dose of 105mg PZQ/kg BW/day. An efficacy trial by Partridge et al. (2012) shows that PZQ inclusion of 2.5g/kg of feed was sufficient to treat skin and gill flukes in *S. lalandi* under 500g, which is applicable for the trial fish, however in larger fish a higher concentration of dietary inclusion would be required because the fish eat a smaller percentage of their total body weight (up to 16g/kg of feed could be required to administer 65mg/kg/day). To administer this same dose of 105mg/kg BW in a 3kg fish fed 1% BW/day would require a dietary inclusion level of 10.5g PZQ/ kg, a much larger concentration than tested in this study.

It was not possible to further limit the size variation, which may have been a source of standard error throughout the trial. A larger population from which to select trial individuals would have been optimal. The trial fish then had to be acclimated to a new habitat and feed regimen. The different light hours, tank size and overall population structure may have impacted the overall consumption numbers during the acclimation period as well. The acclimation feeding, however, was long enough that all individuals should have fully adapted to the new environment, and stable feed consumption data showed that.

A final limitation is that no cost analysis was performed to accurately determine the economic benefits of PZQ incorporated into the feed when compared to other treatment alternatives. Simple online research, however, shows that 50g of pure PZQ powder can be obtained $63.96 USD, and liquid garlic extract can be purchased for $65.79-115.15 USD per kilogram. These amounts, even in bulk, are miniscule when compared to the infrastructural, labor and treatment costs of bath treatments.

4.3 Impacts on Aquaculture:

A primary concern with feed rejection is the inability to accurately calculate dose rates, however with good palatability and a known consumption rate and concentration of PZQ in the feed, calculating dose rates should prove to be less problematic. This also improves the overall economics of PZQ as a medication for fish with *B. seriolae* and/or *Z. seriolae* parasitic infections. Less of the drug is wasted due to higher consumption, while increased intake also results in more stable growth rates (Williams et al., 2008). This should also reverse the results witnessed by Williams et al. (2008), where lower PZQ doses (50 and 75 mg kg^{-1} BW d^{-1}) resulted in better removal of skin and gill flukes than higher concentrations (100 and 150 mg kg^{-1} BW d^{-1}) due to increased palatability concerns with higher doses. The fish are also less stressed compared to bath treatments, reducing the potential for secondary infections or other related effects. Another potential issue was the risk of destroying the drug during the extrusion process, however efficacy trials by Partridge et al. (2012) showed no debilitating effects from heat, moisture or pressure.

While the feed is more expensive than a basic pellet diet, PZQ inclusion reduces the labor required to successfully and treat the fish and streamlines the medication process. It also minimizes the impact of external effects, including weather, currents and waves (in the case of sea cage treatment). The medication process can be incorporated into the hatchery or farm routine, requiring slightly more careful observation during the feeding process.

4.4 Future Studies

There are many future trials that should follow the current study to further elaborate on the significance of the results obtained. While the current trial exhibits the significant consumption benefits of surface-coating GB1 at 50ml/kg of feed, a dose of 105mg PZQ/kg BW/day is easier to administer in smaller fish (Hirazawa et al., 2004; Williams et al., 2008; Partridge et al., 2012). An efficacy trial using the concentration provided in the current study should be completed to determine the ability of this feed to eliminate *B. seriolae* and *Z. seriolae* ectoparasitic infections in the fish. The efficacy trials should include both large and small individuals, as the overall feed intake (%BW/day) varies with size, thereby influencing the dose rate and PZQ concentration required in the feed.

Future trials should also incorporate different PZQ concentrations to see if 50ml GB1/kg of feeds would be a sufficient masking agent. Different garlic surface-coating concentrations should be trialed as well to see if more garlic could possibly mask higher PZQ concentrations in the feed. Higher PZQ concentrations would increase economic feasibility, as less feed would be required to deliver sufficient medication.

The current study was limited to juvenile *Seriola lalandi*, however *B. seriolae* and *Z. seriolae* have been observed in a wide array of *Seriola* spp. (Sharp et al., 2003; Tubbs et al., 2005). The current palatability study, as well as efficacy trials, should be expanded to incorporate data from other species, including *S. dumerilii* and *S. quinqueradiata*. This would not only determine whether this specific treatment is effective in other species, but also help to establish a global protocol for treatment against skin and gill flukes in *Seriola*

aquaculture. Future trials could test different PZQ concentrations, GB1 concentrations and different size classes for each of the affected species.

The feeds used in this trial were either manufactured by Skretting or by CSIRO, limiting the scope of the study. While the nutrition department of CSIRO delivered the feed with PZQ inclusion, future trials could examine the potential to use larger pellets (as opposed to the 3mm pellets), focusing both on the effects of the extrusion process as well as the ability to successfully surface coat the feed prior to feeding. These studies could also further examine the salient effects of garlic to determine whether the masking agent is having and direct or indirect effects on the health of the fish.

4.5 Conclusion

This study successfully demonstrated the ability to mask the bitter flavor of praziquantel when used as an in-feed medication against ectoparasites. Garlic paste is clearly suitable as a masking agent, and may demonstrate some salient effects of its own when fed to fish in an aquaculture system. These findings enhance the logistic and economic capacity of praziquantel use as a medication and provide a framework upon which to perform future relevant trials and create a comprehensive treatment protocol for *Seriola* spp. aquaculture.

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