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Effects of turbidity on the aerobic physiology and feeding behaviour of juvenile snapper (*Pagrus auratus*)

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A thesis submitted in partial fulfilment of the requirements for the degree of Masters of Science in Marine Science

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ABSTRACT

Turbidity as a result of increased suspended sediment in coastal waters is an environmental stress of worldwide concern. Recent research on fish suggests that detrimental changes to gill structure can occur in turbid waters, with speculation that these alterations diminish fitness variables such as growth and development by negatively impacting the O$_2$ uptake capacity (respiration) of fish. To specifically address this unknown, the impact of turbid water on the gill structure, somatic growth rate and O$_2$ uptake rates of a juvenile sparid species (*Pagrus auratus*) was addressed following exposure to 5 different turbidity treatments (<10, 20, 40, 60, 80 NTU) for 30 days. Significant gill structural change was apparent with a progressive increase in turbidity and was quantified as a reduction in lamellae density, as well as increase in basal hyperplasia, epithelial lifting and increased oxygen diffusion distance across the lamellae. The weight of control fish did not change but all fish exposed to turbid waters lost weight, confirming that long term turbidity exposure is detrimental to growth, productivity and fitness. However, the hypothesis that structurally altered gills would impair O$_2$ uptake was not supported due to no measurable difference in the standard metabolic rate (SMR), maximum metabolic rate (MMR), aerobic metabolic scope (AMS) or critical oxygen saturation ($S_{crit}$) limit of fish between the 5 NTU treatments. The results therefore suggest that *P. auratus* may be more resilient to turbidity stress than previously assumed, possibly because they maintain excess gill structure under non-turbid conditions to safeguard O$_2$ supply. To further investigate the reasons behind the observed growth deficit with turbidity exposure, the feeding performance of *P. auratus* was also examined under the same 5 turbidity treatments. Significant reductions in feeding ability were apparent with a progressive increase in turbidity, and was quantified as a reduction in attack success, foraging bites and attack distance. These results suggest that the ability of *P. auratus* to feed effectively and efficiently may impact on fish
growth, however there is likely to be more than one mechanism at play which, as discussed, provides considerable scope for further research.
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### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AMS</td>
<td>Aerobic metabolic scope</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ET</td>
<td>Epithelial tissue region</td>
</tr>
<tr>
<td>FL</td>
<td>Fork length</td>
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<tr>
<td>L</td>
<td>Lamellae area</td>
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<tr>
<td>MMR</td>
<td>Maximum metabolic rate</td>
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<tr>
<td>$\dot{MO}_2$</td>
<td>Mass specific rate of oxygen consumption</td>
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<tr>
<td>NT</td>
<td>Non-tissue space</td>
</tr>
<tr>
<td>NTU</td>
<td>Nephelometric turbidity units</td>
</tr>
<tr>
<td>O$_2$</td>
<td>Oxygen</td>
</tr>
<tr>
<td>PS</td>
<td>Pillar system</td>
</tr>
<tr>
<td>$S_{\text{crit}}$</td>
<td>Critical oxygen saturation</td>
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<tr>
<td>SGR</td>
<td>Somatic growth rate</td>
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<td>SMR</td>
<td>Standard metabolic rate</td>
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1 INTRODUCTION

1.1 ENVIRONMENTAL CHANGE

The growth of human populations is placing an ever-increasing demand on terrestrial and aquatic systems. Land based activities such as land clearance, urbanization, forestry and agriculture have serious direct impacts of their own that are readily observable. However, anthropogenic activities on land also have knock-on effects in the marine environment, particularly coastal waters. These disturbances are perhaps less well studied, or even noticeable, as land based changes, and include increases in sedimentation and turbidity (Hayward et al., 2006), pollution, degradation of water quality, eutrophication (Smith et al., 1999), loss of habitat and anthropogenic climate change (Harley et al., 2006). Although New Zealand is comparatively a “young” country with regards to human occupation, the intensive use of the land by humans, particularly the development of coastal towns and cities, has meant that New Zealand has not escaped environmental changes and degradation (Morrison et al., 2009). Activities such as dairying and forestry that support our production-based economy have resulted in significant impacts for adjacent coastal marine systems (Morrison et al., 2009). Of particular relevance to this thesis are the impacts of suspended sediment that render aquatic environments turbid.

1.2 WHAT IS TURBIDITY?

Suspensoids are defined by Bruton (1985) as “solid or colloidal particles which are held in suspension in a liquid”. There are three primary ways in which suspensoids are measured in
the water column: water clarity, total suspended solids and turbidity. Although these three metrics all provide a measurement of suspended sediment, they differ in the aspects of suspended sediment that they calculate. Water clarity is a direct measure of visible distance through the water column and is a traditional method measured using a black and white Secchi disk submerged into the water until it is no longer visible (Davies-Colley and Smith, 2001). Total suspended solids (TSS) represents an actual measure of organic particles suspended in the water column using dry weight measurements of suspended sediment, typically reported as mg/l. The concept of turbidity is, in simple terms, the ‘cloudiness’ of the water, and results from intense scattering and absorption of light by fine particles in the water column (Kirk, 1985) such as silt, clay, organic and inorganic matter, plankton and other microscopic organisms (Bash et al., 2001, Bruton, 1985). As such, waters with high concentrations of fine suspended sediment are described as ‘turbid’ and inevitably these waters are of low visual clarity. Turbidity is measured in nephelometric turbidity units (NTU), which is the relative measurement of light scattering through a restricted range of angles to the incident light beam (Davies-Colley and Smith, 2001). Although turbidity cannot be directly correlated with actual suspended sediment concentration in the water, it is used as in indicator of suspended sediment levels as opposed to a direct measure (Bash et al., 2001). Nevertheless, turbidity is currently widely used for the monitoring of suspended sediment in marine and freshwater systems alike. The present study uses controlled laboratory-based experimentation to assess the effects of suspended sediment on fish using known quantities of sediment added to the water. Therefore, for the purpose of this study, turbidity is an effective method for measuring suspended sediment concentrations in the water column. From this point on, suspended sediment and turbidity will be used interchangeably throughout this thesis.
1.3 TURBIDITY WORLDWIDE AND IN NEW ZEALAND

Suspended sediment is now recognised as a major environmental stressor of worldwide concern (Airoldi, 2003, Gray, 1997) and is arguably already causing significant degradation of aquatic ecosystems both worldwide and in New Zealand. There are countless examples in the literature that highlight the significance of turbidity as an increasing environmental stressor worldwide. For example, changes in sedimentation have been dramatic in some coral reef systems, with negative effects on coral richness on the Great Barrier Reef (Fabricius and De’ath, 2001). Similarly Hewawasam et al. (2003) showed that the conversion of forest to agricultural land in Sri Lanka has increased the rate of sediment run off from 13-30 metric tons per km² per year to up to 7000 metric tons per km² per year. Furthermore, the average sedimentation rate in Chesapeake Bay has increased by orders of magnitude since the first land clearing activities were initiated in 1760 (Cooper and Brush, 1993). The increasing concern regarding degradation of coastal habitats through increases in sedimentation is reflected in the volume of research that has been directed towards this problem in recent years. As further human population growth and subsequent development is projected to increase, understanding and predicting effects of suspended sediment on coastal ecosystems is fundamental to sustainable management.

Sediment input to the coastal marine environment depends on the characteristics of the land, such as soil erodibility, amount of rainfall and the presence or absence of rivers (Morrison et al., 2009). In light of this, the New Zealand terrain is characteristically hilly with steep catchments and many areas are composed of highly erodible soils such as soft siltstones/mudstones, particularly in north eastern New Zealand (Griffiths and Glasby, 1985, Morrison et al., 2009). These characteristics, along with New Zealand’s short history of colonisation and consequent rapid change in land use, are responsible for a very pronounced
increase in sedimentation rates in the coastal marine environment (Morrison et al., 2009). Although the erosion of soil and its transportation to the coast is a natural process, vast quantities of soil have been mobilised as a result of land-use changes such as extensive deforestation throughout the country (Morrison et al., 2009). This soil is ultimately transported to the coast and thus, high suspended sediment loads are now characteristic of many estuaries and coastal zones in New Zealand (Morrison et al., 2009). For example, in the Mahurangi harbour 3 meters of sediment has accumulated since deforestation in the 1900’s (Swales et al., 2002).

The input of sediment to the coastal zone of New Zealand is now particularly high by world standards, close to almost 1 % of total world sediment yields (Morrison et al., 2009, Robertson and Stevens, 2006). With the predicted increase in both intensity and frequency of rain and storm events associated with climate change (Willis et al., 2007), the level of sedimentation in both freshwater and marine environments is only expected to increase (Morrison et al., 2009, Willis et al., 2007). Whilst suspended sediment measures are an important parameter in environmental impact assessments throughout the world (Gray, 1997, Morrison et al., 2009, Scarsbrook, 2008), understanding the exact biological impacts of suspended sediment is crucial for effective ecosystem management.

1.4 BIOLOGICAL EFFECTS OF TURBIDITY

Increased sediment runoff can elicit a wide range of effects in the marine environment from deposition (smothering) on the sea floor to increased suspended sediment loads in the water column that renders the water turbid. Ongoing re- suspension and deposition events (e.g. by storms), can also regularly shift sediments between these two states (Bruton, 1985). The diagram below (Fig. 1.1), taken from Bruton (1985), effectively illustrates the array of
biological effects of sediment in the water column and may be summarized as follows:
Scattering and absorption of light reduces light penetration in the water column (Bash et al., 2001), which decreases the light available for photosynthesis for phytoplankton, macrophytes (Bruton, 1985) and coral reef systems alike (Fabricius, 2005), altering the production and distribution of these organisms (Jones, 2008, Nichlolls et al., 2003). This can have flow on effects for higher trophic levels through changes in food availability for many benthic invertebrates and herbivorous fish species (Bruton, 1985, Jones, 2008). Blanketing of benthos through deposition of suspended sediment has similar effects as described in the previous paragraph but can also elicit negative impacts on benthic invertebrates through smothering of organisms and clogging of feeding apparatus (see review by Morrison et al., 2009). This can cause reductions in feeding and increased susceptibility to disease (Morrison et al., 2009, Newcombe and MacDonald, 1991). Light attenuation by suspended sediment also diminishes water clarity, reducing visual acuity of fish and hence the ability of visual predators to feed (Bruton, 1985, Davies-Colley and Smith, 2001). Suspended sediment can also yield other important effects on fish, in particular through gill clogging, direct gill damage and other associated indirect impacts (Bruton, 1985, Lowe et al., 2015). The last two effects described in this summary specifically address impacts on fish. These two impacts (gill clogging/damage and reduced visibility for feeding) are the foundations of this thesis and are addressed in more detail in the following sections.
Figure 1.1 Conceptual overview outlining the array of effects of suspended sediment in the marine environment. Diagram taken from Bruton (1985).
1.5 **GENERAL EFFECTS OF TURBIDITY ON FISH**

An increase in frequency and intensity of suspended sediment is shown to have direct and indirect impacts on fish populations. These effects can be pronounced, including loss of important nursery habitat such as sea grass beds (Morrison *et al.*, 2009), reductions in visibility and foraging ability (Johansen and Jones, 2013) and gill clogging/damage (Lowe *et al.*, 2015). Much of the knowledge surrounding the effects of suspended sediment on fish is based mainly on salmonid species, however a number of studies in recent years have contributed to the knowledge of marine fish species and their responses to sediments. In order to describe the effects of suspended sediment on fish in greater detail, both freshwater and marine fish species examples are used throughout this thesis. Although responses of marine and freshwater species to turbidity may differ this is also the case when comparing among marine species. Therefore, the examples that follow encompass the wide range of effects that can be seen in fish as a result of turbidity exposure.

1.5.1 **Impacts of turbidity on fish gills**

*Direct impacts* of suspended sediment on fish gills are relatively well covered in the literature and there is now clear evidence that structural changes to fish gills do occur as a result of increased suspended sediment (Au *et al.*, 2004, Hess *et al.*, 2015, Lowe *et al.*, 2015, Wong *et al.*, 2013). Morphological changes seen in gill structure as a result of suspended sediment exposure are thought to be a protective mechanism to safeguard the inner gill tissues (pillar system) from particulate abrasion (Mallatt, 1985). These changes are commonly observed in the form of epithelial hyperplasia (Hess *et al.*, 2015, Lowe *et al.*, 2015), lamellar fusion (Lowe *et al.*, 2015, Wong *et al.*, 2013), hyperplasia at the base of lamellae (Wong *et al.*, 2013), epithelial lifting (Au *et al.*, 2004, Wong *et al.*, 2013) and hyperplasia of the pillar system (Au
et al., 2004), which all collectively increase the oxygen diffusion distance across the secondary lamellae (Hess et al., 2015).

**Indirect impacts** of turbidity on fish gills such as alterations in metabolism, respiration and fish fitness are perhaps less well studied. Whilst it would be logical to assume that altered gill structure (as above) would indirectly impact the efficiency of oxygen transfer from water to blood across the gills (as has been suggested by a number of authors), this has not actually been established. For example, Wenger et al. (2014) observed a slow rate of development in clownfish larvae exposed to suspended sediment and this was suggested by Hess et al. (2015) to be a consequence of respiratory stress caused by a drastic change in gill morphology. However, this conclusion was only based on evidence of gill damage, not measures of respiration per se. Similarly, Lowe et al. (2015) also presented evidence of an increase in ventilation with progressive turbidity but, even though this single measure cannot accurately signal a change in oxygen uptake, it was speculated that the respiratory impact of impaired gill function was associated with the poor growth performance of their fish. Likewise, the study of Wong et al. (2013) reported impacts on respiration as a result of sediment-induced gill damage but, again, although the term “respiration rate” was used, changes in the transfer of oxygen across the gills were not documented (Wong et al., 2013).

### 1.5.2 Impacts of turbidity on fish visual performance

**Direct impacts** of turbidity on fish visual performance is perhaps rather obvious, where light attenuation by suspended matter in the water column effectively reduces the visual range of fish (Utne-Palm, 2002). The visibility of a prey item requires the predator to detect a difference in contrast between the background and the prey item (Utne-Palm, 2002). Therefore the ability of fish to detect prey can be limited by the optical environment. As such, suspended particles in the water column scatter and absorb light and decrease contrast and light penetration, thereby
interfering with the visual acuity of fish (Utne-Palm, 2002). The visual range of fish has been measured in various ways, but in most cases, visual range can be determined by fish behaviour. A common measure is reactive distance, which is the distance at which the test subject may react to a visual stimulus in its environment (Utne-Palm, 1999). This distance is commonly influenced by light levels, i.e. turbidity (Meager et al., 2005, Utne-Palm, 1999) and is expressed in their behaviour as outlined below.

**Indirect impacts** of turbidity on fish visual performance include changes in ability to detect and capture prey items with observed reductions in attack rate and feeding success. Many studies demonstrate detrimental effects of suspended sediment on the foraging rate of fish, including increases in reaction distance (Utne-Palm, 1999), reductions in number of feed items consumed (Hasenbein et al., 2013, Wenger et al., 2012) and increased time taken to find food (Wenger et al., 2012). For example, turbidity levels of just 4 NTU decreased foraging rates, and average attack success was reduced by up to 56 % in planktivorous coral reef fish (Johansen and Jones, 2013). Similar trends are seen in freshwater fish species where suspended sediments were found to have a negative impact on feeding behaviour (feeding rate and reaction distance) of both turbidity tolerant and intolerant freshwater fish species (review by Chapman et al., 2014). For example, coho salmon demonstrated a significant decrease in reaction distance to prey, capture success and the percentage of prey ingested in turbid water conditions (30 and 60 NTU) (Berg and Northcote, 1985). Nevertheless, not all fish respond negatively to suspended sediment. Many fish thrive in turbid environments as low levels of suspended sediment can enhance visual contrast of prey items, effectively increasing overall feeding rates (Morrison et al., 2009) as well as reducing risk of predation for some species resulting in increased foraging rates as seen in juvenile chinook salmon (Gregory and Northcote, 1993).

Indirect impacts of changes in feeding ability are perhaps more difficult to study than direct impacts. However, it is commonly thought that reductions in prey acquisition through a
decrease in visual acuity can affect the growth, survival and fitness of fish. For example, coral reef fish exhibited prolonged larval development under turbid conditions (Wenger et al., 2014) and increased suspended sediment reduced the growth and condition of planktivorous damselfish at relatively low levels (Wenger et al., 2012). Unfortunately, there is limited literature that explores the direct cause of growth deficits in fish exposed to turbidity.

1.6 AIMS AND HYPOTHESES

The effects of turbidity on fished species is a growing field of research. Recent research efforts describe effects on coral reef fish species and freshwater species, but the research by Lowe et al. (2015) is the only study to address the effects of turbidity in a New Zealand marine fish of commercial and recreational importance. Due to increased levels of suspended sediment in the New Zealand marine environment, (and predictions for this to deteriorate (Morrison et al., 2009, Willis et al., 2007)), surprisingly little is known regarding the effects on ecologically and commercially important demersal fish species. It is understood that suspended sediments affect the gill structure of fish but it has only been speculated that changes in gill structure affect the efficiency of oxygen transfer from water to blood across the gills, leading to respiratory stress and reductions in performance (Hess et al., 2015, Lowe et al., 2015, Wong et al., 2013). This has yet to be tested directly. Although decreased oxygen consumption has been logically assumed to be associated with poor growth performance in a number of fish species, it is possible that these growth deficits observed across multiple studies may also be at least partly, explained by a reduction in visual feeding performance with increasing turbidity. Clearly, further investigations are required.
Chapter 1

The overall purpose of this thesis was to investigate the oxygen uptake ability of fish post turbidity exposure and to quantify changes in growth, gill structure and feeding behaviour. These findings are important for developing an understanding of turbidity impacts at a physiological and behavioural level, and to develop a greater understanding for more effective management and monitoring of turbidity in a New Zealand marine context. This was achieved through two main experiments, which comprise Chapters 2 and 3 in this thesis.

1.6.1 Experiment 1

The first experiment (Chapter 2), aims to address a big knowledge gap regarding the ability of fish to take up oxygen across damaged gills following exposure to suspended sediment. To address this unknown, respirometry methodology was utilised following exposure to varying levels of turbidity. The main objectives here were to:

- Quantify changes in gill structure following exposure to graded levels of turbidity.
- Investigate changes in oxygen uptake to examine gill oxygen uptake function as a result of turbidity exposure.
- Assess whether the influence of graded levels of turbidity exposure also has an impact on fish growth, potentially via a change in oxygen uptake.

It was hypothesised that fish would exhibit significant changes in gill structure with increasing turbidity exposure as seen in previous studies. Consequently it was hypothesised that oxygen uptake efficiency would be reduced as a direct result of increased oxygen diffusion distance across the lamellae. It was further expected that this hypothesised reduction in oxygen supply to the tissues would result in a decline in somatic growth rate.
1.6.1.1 Methodology

To address these questions, established histological methods were utilised to analyse changes in gill structure and standardised closed-system intermittent respirometry methods were applied to calculate oxygen uptake (Svensden et al., 2016). Respirometry, although it is standard methodology, requires a comprehensive explanation of the measurements that are involved in order to effectively understand the methodology used in Chapter 2. Respirometry is a well-recognised method for measuring oxygen consumption rate ($\dot{MO}_2$) in fish and from this a number of metabolic measures can be calculated (outlined in figure 1.2) (Nelson, 2016). This includes the standard metabolic rate (SMR) as the best estimate of basal metabolic rate which is the lowest amount of energy consumption required to maintain the function of an organism at rest (Chabot et al., 2016, Domenici et al., 2013, Schurmann and Steffensen, 1997).

Maximum metabolic rate (MMR) on the other hand describes the maximum rate of oxygen consumption of a fish and is typically measured using maximum rates of oxygen consumption during or following intense exhaustive exercise (Domenici et al., 2013, Norin and Clark, 2016). Aerobic metabolic scope (AMS) therefore, is the difference between SMR and MMR and is a measure of the metabolic scope for non-essential activity of the organism (Chabot and Claireaux, 2008, Chabot et al., 2016, Fry, 1971). Put another way, AMS represents the amount of oxygen which can be supplied for routine activities such as swimming, growth and reproduction (Farrell and Richards, 2009) and represents the metabolic limits within which all aerobic activities must be undertaken (Chabot and Claireaux, 2008, Fry, 1971) and is effectively reduced (compressed) during hypoxia (Fig. 1.2). Species that are able to maintain a largely constant SMR over a wide range of ambient oxygen saturations are referred to as oxygen regulators (McKenzie et al., 2007, Schurmann and Steffensen, 1997, Steffensen, 1989). However, the point where SMR “breaks” and fish transition from being an oxygen regulator to an oxygen conformer is known as the critical oxygen saturation ($S_{crit}$) level (Schurmann and
Steffensen, 1997) (Fig. 1.2). At this point AMS is zero and standard metabolism is reduced (i.e. conforms to the external environment) as oxygen levels are lowered further. As such, fish that remain in low oxygen environments (hypoxia) below their $S_{\text{crit}}$ cannot meet their entire aerobic oxygen demand and will eventually asphyxiate once anaerobic energy resources have been consumed (Schurmann and Steffensen, 1997). In this way hypoxia exposure and the metabolic measures that can be calculated from this, provide an effective means of testing the performance of damaged gills as a result of turbidity exposure.

Figure 1.2 Conceptual overview of the metabolic responses of fish to hypoxia. MMR = maximum metabolic rate, SMR = standard metabolic rate, AMS = aerobic metabolic scope, $S_{\text{crit}}$ = critical oxygen saturation levels where SMR is equal to MMR. Concepts taken from Fry (1947), Schurmann and Steffensen (1997) and Cook et al. (2011).
1.6.2 Experiment 2

The second experiment (Chapter 3), aims to assess the feeding behaviour of fish during exposure to increasing levels of turbidity using an experimental swim flume. The main objectives here were to:

- Investigate changes in ability to successfully capture prey items under flow in turbid conditions.
- Investigate changes in reaction distance to prey items under flow in turbid conditions.

It was hypothesised that fish experiencing a progressive increase in turbidity, would demonstrate a significant reduction in successful prey capture and show an increased reaction distance to prey. This hypothesised reduction in feeding ability is proposed as a complementary hypothesis to that in Chapter 2, where reductions in feeding ability is thought to further contribute to reductions in growth observed with exposure to turbidity.

1.7 Study species

Breams (*F. Sparidae*) are widely distributed fish across the continental shelf and reside in temperate waters throughout the South Pacific including the Philippines, Indonesia, Taiwan, China, Japan (Parsons et al., 2014), New Zealand, Australia and Norfolk Islands (Paulin, 1990). The New Zealand snapper (*Pagrus auratus*) is not a true snapper (*F. Lutjanidae*) but is a member of the bream family *Sparidae* and is one of the most sought after inshore species in northern New Zealand waters, and are therefore a highly valuable fished species both recreationally and commercially (Willis et al., 2003). Valuable fisheries are also located in Japan and Australia (Paulin, 1990). This species plays a significant role in the culture of New Zealand, providing food and recreation (Parsons, et al., 2014), as well as generating significant economic activity, with an estimated commercial catch of 3456 tonnes during the 2011-12
fishing year (Hartill et al., 2013). *P. auratus* are also ecologically important, contributing a large proportion of fish biomass in northern New Zealand (Francis, 1997). They occur in association with both hard and soft substrates systems. However, juveniles are found to be strongly associated with shallow sheltered waters such as estuaries, harbours and coastal embayments (Hartill et al., 2003, Langley, 1993), highlighting the importance of these habitats as nursery grounds. The degradation of these sheltered coastal systems due to the steady increase of human pressures has been widely documented (Morrison et al., 2009, Nixon, 1995, Smith et al., 1999, Wu, 2002). Therefore, due to the reliance of *P. auratus* on these coastal habitats, it is important to quantify any effects that may occur as a result of habitat and water quality degradation.

*P. auratus* was selected as a model species for this study for two reasons: 1) The issue of increasing suspended sediments is of utmost relevance to this recreationally and commercially fished species in New Zealand because it is abundant in the Hauraki Gulf where water turbidity is increasing at the highest rate in New Zealand due to city expansion and land-use change (Morrison et al., 2009). 2) This study also builds on the body of work that documents the oxygen consumption rate of this species to hypoxia (Cook and Herbert, 2012, Cook et al., 2013, Cook et al., 2011) which effectively allows us to cross reference existing data against the oxygen consumption rate of fish in this study for the purpose of data validation.

Both small and large juvenile *P. auratus* tend to occur in greatest numbers in shallow coastal and estuarine habitats (Parsons et al., 2014). Studies analysing the chemical signatures within the otoliths of *P. auratus* suggest that adult snapper from large spatial ranges originate from only one or two nursery habitats within that range (Fowler et al., 2005, Hamer et al., 2011, Morrison et al., 2009). For example, Morrison et al. (2009) demonstrated that the majority of adult *P. auratus* from the west coast North Island population (SNA 8) most likely originate from the Kaipara Harbour as small juveniles. These results illustrate that for the North
Chapter 1

Island of New Zealand it is likely that a few critical estuarine habitats have disproportionate value in the maintenance of adult *P. auratus* populations. This information is crucial as these estuarine and shallow coastal habitats in which these juvenile *P. auratus* reside are where sedimentation effects are the greatest (Morrison *et al.*, 2009). Increased sedimentation and turbidity may therefore serve as a potential bottleneck for *P. auratus* populations in northern New Zealand.

Long term turbidity monitoring is carried out by the Auckland Council and provides useful information regarding levels of suspended sediment in locations that may serve as key habitats for *P. auratus*. For example in the Kaipara harbour, which is an important nursery habitat for *P. auratus* (Morrison *et al.*, 2009), 7 monitoring sites have been established (Scarsbrook, 2008). The raw data from 2009 - 2014 for these sites show that turbidity can range from 0.2 NTU to as high as 150 NTU with averages between 2.3 and 26.5 NTU within this estuary (Table 1.1). This data is of particular importance as it provides a reference with which to assess the ecological significance of thresholds obtained in this study. It must be noted, however, that these data are but a snapshot in time as all values are spot readings taken monthly at single specific locations (Scarsbrook, 2008). Additionally due to the conditions in which high turbidity often occurs, samples may not have been taken during high turbidity conditions for health and safety reasons. These results must therefore be interpreted with caution.

To conclude, given the significance of turbidity as a stressor in the NZ marine environment, and the importance of *P. auratus* as a commercial and recreational fish species, it is hoped that this thesis will provide valuable insight into some of the indirect physiological and behavioural impacts of turbidity and will hopefully fill a knowledge gap surrounding the mechanisms behind the observed growth deficit in fish when exposed to suspended sediment. Such findings could provide scientific knowledge to inform management and monitoring of turbidity in New Zealand.
Table 1.1: Turbidity (NTU) data collected by the Auckland Council from 2009 – 2014 for sites in the Kaipara harbour.

<table>
<thead>
<tr>
<th></th>
<th>Kaipara Heads</th>
<th>Omokoiti Beacon</th>
<th>Shelly Beach</th>
<th>Kaipara River</th>
<th>Makarau Estuary</th>
<th>Hoteo River</th>
<th>Tauhoa Channel</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Average NTU</strong></td>
<td>2.3</td>
<td>3.9</td>
<td>26.5</td>
<td>20.3</td>
<td>8.6</td>
<td>6.6</td>
<td>2.8</td>
</tr>
<tr>
<td><strong>Minimum NTU</strong></td>
<td>0.6</td>
<td>0.3</td>
<td>0.2</td>
<td>3.3</td>
<td>1.9</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>Maximum NTU</strong></td>
<td>8.6</td>
<td>12.0</td>
<td>150.0</td>
<td>55.1</td>
<td>31.7</td>
<td>23.4</td>
<td>8.9</td>
</tr>
</tbody>
</table>
2  **EFFECTS OF TURBIDITY ON THE AEROBIC PHYSIOLOGY OF JUVENILE SNAPPER (*PAGRUS AURATUS*)**

2.1 **INTRODUCTION**

Anthropogenic activities play a major role in increasing the rate of sediment run off from land that renders coastal environments turbid (Morrison *et al.*, 2009). This worldwide phenomenon appears to be escalating but managing the problem might be facilitated if it is more precisely understood how turbid waters affect marine biota. An increase in the frequency and intensity of suspended sediment is shown to have direct and indirect impacts on fish populations. For example, fish exposed to elevated levels of suspended sediment show signs of gill damage following direct contact with sediments in the water (e.g. Au *et al.*, 2004, Hess *et al.*, 2015, Lowe *et al.*, 2015) but indirect impacts are also apparently accrued in the form of low rates of feeding, reduced growth, delayed maturation and increased susceptibility to disease (Bruton, 1985, Newcombe and MacDonald, 1991, Sutherland and Meyer, 2007).

Understanding the link between direct and indirect impacts of suspended sediment is important as it allows a full understanding of cause and effect and therefore the ecosystem changes likely to occur as result of increased sediment loads. In this regard, fish gills are very important to consider because they are in direct contact with the environment and are highly vulnerable as they present fragile and exposed membranes, vital for the transfer of gases, ions and nitrogenous compounds between the body and environment (Evans *et al.*, 2005). As outlined in Chapter 1, there is now clear evidence that structural changes to fish gills do occur as a result of increased suspended sediment (Au *et al.*, 2004, Hess *et al.*, 2015, Lowe *et al.*, 2015).
Chapter 2

2015, Wong et al., 2013). The changes outlined in Chapter 1 are thought to cause decreased growth and development in fish following turbidity exposure due to a decreased ability to take up oxygen across damaged gill tissue (Hess et al., 2015, Lowe et al., 2015, Wong et al., 2013). Although these speculations are entirely logical, to prevent speculation from being misconstrued as fact in future reports, it is now important to ascertain whether damage to the gill lamellae as a result of suspended sediment exposure does indeed reduce the capacity for oxygen transfer and lead to respiratory stress and reductions in performance as suggested (Hess et al., 2015, Lowe et al., 2015, Wong et al., 2013). This is an important direction for turbidity-related research because an adequate oxygen supply is vital in setting the limit to important bodily functions such as growth (Claireaux and Lefrançois, 2007), reproduction (Holt and Jørgensen, 2015), intra-specific interactions (Biro and Stamps, 2010) and locomotion (Metcalfe et al., 2016, Schurmann and Steffensen, 1994). Oxygen uptake thus appears integral to the fitness of different organisms in different environments (Biro and Stamps, 2010, Claireaux and Lefrançois, 2007, Holt and Jørgensen, 2015, Metcalfe et al., 2016).

To address the link in direct and indirect impacts of turbidity exposure, this study specifically set out to test whether fitness variables such as growth are negatively impacted by gill structural change and a limitation of O₂ uptake capacity (respiration) in fish. A juvenile sparid fish species (the New Zealand snapper, Pagrus auratus) was therefore exposed to 5 different levels of water turbidity (< 10, 20, 40, 60, 80 NTU) over a 30 day period, after which their gill structure and various measures of oxygen consumption were examined. Established techniques in intermittent flow-through respirometry were used to compare the oxygen uptake rates of P. auratus across the 5 turbidity treatments. This involved measuring and comparing the standard metabolic rate (SMR), maximal metabolic rate (MMR) and aerobic metabolic scope (AMS) of fish under well oxygenated conditions, in addition to the critical oxygen saturation (S crit) limit of fish under low O₂ (i.e. hypoxic) conditions.
Chapter 2

*P. auratus* have been shown to develop progressive gill structural change with increasing levels of turbidity (Lowe *et al.*, 2015) but this study attempts to measure the physiological impact of this change with a specific set of hypotheses. Whilst general disturbances to oxygen uptake efficiency is speculated by others (Au *et al.*, 2004, Hess *et al.*, 2015, Lowe *et al.*, 2015, Wong *et al.*, 2013), our specific hypothesis is based on the results of Cook, *et al.* (2011) who showed that anaemic *P. auratus* with a reduced blood oxygen carrying capacity experienced a reduction in both MMR and AMS (but not SMR, see below) and so were forced to avoid hypoxia earlier because their $S_{\text{crit}}$ limit was raised. The following set of specific hypotheses were therefore developed: 1) There is evidence that cardio-ventilatory adjustments can maintain the routine metabolic function of stressed fish (Bindon *et al.*, 1994a, Bindon *et al.*, 1994b, Chabot *et al.*, 2016) so it is feasible that SMR (as the best experimental estimate of basal ‘maintenance’ metabolism) would either not change as a result of turbidity-related gill change or would increase slightly if compensatory adjustments add to the SMR costs of maintenance. There is no expectation that SMR would be reduced unless cardiovascular adjustments are unable to compensate for reduced $O_2$ carriage across seriously impaired gills. However, 2) because MMR and AMS represent peak aerobic function, these measures should be reduced if gill structural change as a result of turbidity exposure does indeed limit oxygen supply. Similarly, 3) $S_{\text{crit}}$ represents the $O_2$ conforming breakpoint of fish under challenging low $O_2$ conditions so would be expected to increase if gill structural change does indeed impair oxygen supply across the gills.
2.2 MATERIALS AND METHODS

2.2.1 Fish handling and treatment

Approximately 100 juvenile New Zealand snapper (*P. auratus*, Sparidae) ranging in weight from 15-60 g and fork length (FL) from 88-125 mm were obtained from the Plant and Food hatchery in Nelson and transported to the Leigh Marine Laboratory. They were housed indoors in three 500 L flow-through black PVC seawater tanks at ambient sea temperature with natural light penetration through sky lighting. Fish were held for a minimum of 4 weeks to allow recovery from any stress associated with transport and fed daily on dry commercial fish pellets (Economy floating fish pellets, Aqua One, P.R.C) at a rate of 3 % body weight per day (BW d\(^{-1}\)). Prior to experimentation fish were tagged with electronically readable PIT (passive integrated transponder devices) implants to allow individual identification before being transferred to treatment tanks. Tagging was carried out under anaesthetic (AQUI-S NZ Ltd, Lower Hutt, New Zealand) and tags were inserted intraperitoneally. Fish were weighed prior to experimentation and again at the end, allowing calculation of individual weight-specific growth rate (SGR, % body weight day\(^{-1}\)) as follows:

\[
SGR = \frac{\ln m_2 - \ln m_1}{t_2 - t_1} \times 100
\]

where, \(m_1\) is the initial weight at the start of the growth period \(t_1\) and \(m_2\) is the final weight at the end of the growth period \(t_2\).

Measurements of turbidity were used in this study as a proxy for suspended sediment and this was reported in nephelometric turbidity units (NTU). A closed system method was used to keep sediment levels constant and continuously suspended over time in five 150 L circular treatment tanks (Fig 2.1). This was achieved by pointing the outlet of two submersible pumps (EHEIM compact 600 and 300, Germany) 2 cm away from the bottom of the tanks so
that water circulated down and around the tank and was held in continual motion (Fig 2.1). A circular bubbler pipe was also laid around the bottom edge of each tank to provide aeration but this also helped prevent sediment settling out around the tank margins. Although sediment was not added to a non-turbidity control tank, this tank was equipped with the same array of pumps and bubblers.

**Figure 2.1** Schematic diagram showing the turbidity tank set up. P represents submersible pumps with red arrows demonstrating water flow from these pumps. The black solid line around the base of the tank represents the circular bubbler pipe to provide aeration. The blue arrows depict water flow.
Five individual *P. auratus* were randomly assigned to each of the 5 treatment tanks and exposed to one of four turbidity treatment levels (20, 40, 60, 80 NTU) or a control (< 10 NTU) for 30 days. These levels were chosen as representative of the turbidity range that these species may encounter in the environment (Table 1.1). A maximum of 5 fish were added so that fish density was kept as low (2.3 kg m$^{-3}$) and water quality as high as possible in the closed system tanks. A low fish density also helped to minimise harmful competitive interactions between conspecifics. The addition of fish to the 5 treatment tanks was replicated temporally three times. The start time of each treatment was also staggered by 4 days to allow time for respirometry ensuring that the duration of turbidity exposure was at least 30 days but no more than 34 days for each group. NTU treatments were also randomly assigned to each tank and between each replicate to safeguard against tank effects. At the start of each replicate, fish were randomly placed into turbidity treatment tanks at 18.0 °C and allowed to acclimatize to the tank for at least 48 hours. Fish assigned to the < 10, 20, 40, 60, 80 NTU treatments had a starting weight of (average ± SEM) 77.57 ± 6.82 g, 70.26 ± 7.79 g, 82.51 ± 6.22 g, 84.23 ± 6.63 g, 81.63 ± 7.77 g respectively, which were not significantly different from each other (1 Way ANOVA. F = 0.325, P > 0.05). Pumps were then switched on for a second acclimation period of at least 48 hours before sediment was added. Due to the natural variation of seawater used in the closed-system tanks, the control tank was labelled < 10 NTU. However, almost all daily measures were below 5 NTU.

Surficial (1 cm deep) estuarine sediment was collected from the upper reaches of the Whangateau estuary (36°18'31.5"S, 174°46'46.9"E) to create turbidity. Sediment was wet sieved down to < 63µm and left to settle overnight. The clear top water was siphoned off and sediment was refrigerated and stored for use. This process was replicated a number of times throughout the experimental duration to provide fresh sediment. Sediment was added to the tanks as a slurry and levels were adjusted by adding varying amounts of stock solution to
achieve the intended turbidity level. Turbidity was monitored daily using a TSS portable handheld measurement instrument (HACH, Germany) and small amounts of extra sediment was added if required. Due to the closed nature of the tanks, total ammonia was monitored daily using an ammonia testing kit and never exceeded 6.0 mg/L and was usually < 1.8 mg/L. Water quality was therefore considered acceptable according to the results of Lemarie et al., (2004) because the growth and survival of juvenile seabass was not affected by 6.6 mg/L of total ammonia after 55 days of exposure. Free ammonia, nitrate and nitrite levels were undetectable. To ensure water quality was maintained long-term across the trial, the 5 tanks were cleaned and flushed every 3-5 days to prevent build-up of toxins, uneaten feed and faeces. Fish continued to be fed daily on pellets at 3 % BW d\(^{-1}\).

2.2.2 Respirometry methodology

After fish were exposed to turbidity treatments for at least 30 days, measures of whole animal oxygen consumption were obtained using automated intermittent flow-through respirometry. Standard metabolic rate (SMR), maximal metabolic rate (MMR), aerobic metabolic scope (AMS) and critical O\(_2\) saturation (S\(_{crit}\)) limits were determined using this method at the Leigh Marine Laboratory according to the methodology of Cook and Herbert (2012), Cook et al. (2011) and Svendsen et al. (2016). Fish were placed into one of two custom-made respirometers that consisted of a chamber (2.4 L or 2.59 L) attached to a flush pump (EHEIM compact 600, Germany) and this entire apparatus was housed in a larger 100 L reservoir that was filled with fresh seawater filtered to 5 µm in each experimental run (Fig. 2.2). The respirometer chambers were filled with clear water so turbidity was not maintained during this part of the experiment. Another external loop of tubing was also attached to each end of the chamber and this contained i) an in-line pump (EHEIM compact 300, Germany) that continually mixed water in the chamber and ii) a cuvette that housed a fibre optic oxygen
dipping probe (OXROB, Pyroscience, Germany) so that oxygen in each chamber could be monitored (Fig. 2.2). Water temperature was held constant at 18.0 ± 0.5 °C mainly because experiments were carried out in a temperature controlled room. However, to safeguard against temperature rising above 18.0 °C, especially in the middle of a hot day, a sea water chiller unit (1200W, ZMT, Germany) equipped with a set of cooling coils in the 100 L reservoir circulated 18.0 °C water at all times to ensure strict temperature control. An extra pump (EHEIM 600, Eheim, Germany) was also used to circulate seawater between the 100 L reservoir and a 40 L oxygenation/ deoxygenation gas tower at a rate of 600 l h⁻¹. Oxygen saturation in the reservoir and respirometry chambers was therefore controlled by bubbling air (for oxygenation) or compressed nitrogen (for deoxygenation) through a bubbler at the bottom of the gas tower. To limit bacterial respiration, seawater circulating between the reservoir and the gas tower was passed through a UV sterilizer (PondOne ClearTec, China). A blackout sheet shrouded the whole set up and was designed to limit external disturbance (Fig. 2.2).
**Figure 2.2** Diagrammatic representation of the respirometry set-up used to measure individual *Pagrus auratus* oxygen consumption at dissolved oxygen concentrations of 100%, 70%, 60%, 50%, 40%, 30%, 25%, 20%, 15%. Blue lines represent water tubing connections and arrows indicate direction of water flow. The diagram shows only one respirometry chamber but two chambers were run at the same time.
After individual fish were sealed in each of the respirometry chambers, a repeating cycle of flush, wait and measure was initiated by customised software operating on a PC notebook (John Atkins, Leigh Marine Laboratory). This software turned the chamber flush pump on (to flush) or off (to wait and measure) for set amounts of time (see below) using a relay control unit (USB Net Power 8800 Pro, Aviosys, Taiwan) connected to the PC. Clean water from the 100 L reservoir was therefore supplied to the chambers when the flush pump was active during the flush period (approx. 2-3 min depending on the size of the fish). Once the flush pump was deactivated a 30 sec wait period ensued to allow for a stable decline in water oxygen saturation due to respiration in the chamber. Thereafter, a 6-9 min measurement phase followed depending on the size of the fish. The decline in chamber oxygen was recorded by the fibre-optic oxygen dipping probe coupled to a Firesting oxygen meter (Pyroscience, Germany) connected to the PC and used to calculate the mass specific rate of oxygen consumption ($\dot{MO}_2$, mg O$_2$ kg$^{-1}$ h$^{-1}$) according to the following equation:

$$\dot{MO}_2 = V(\Delta \% sat / t) \alpha M_n$$

Where $V$ is the respirometry chamber minus fish volume, $(\Delta \% sat / t)$ is the change in oxygen saturation per unit time, $\alpha$ is the solubility coefficient of oxygen (mg O$_2$ % sat$^{-1}$ L$^{-1}$) in water (35 ppt, 18 °C), and $M_n$ is the body mass of the fish in kg (Schurmann and Steffensen, 1997). Fish were transferred in water and introduced to the chambers within 1 min at approximately 16:00 h each day and left undisturbed for approximately 16 h until the next day. In the region of 70-150 $\dot{MO}_2$ cycles were therefore collected from each individual overnight when water was fully air saturated and this data was used to calculate the SMR of each fish under normoxic conditions using the 15 % quantile method of Dupont-Prinet et al. (2010).
Once SMR was resolved the critical oxygen saturation limit ($S_{\text{crit}}$) of fish was assessed by gradually decreasing oxygen in the 100 L reservoir, and thus oxygen in the chambers, in steps (70, 60, 50, 40, 30 25, 20 and 15 % oxygen saturation) until a clearly reduced “break” in $\dot{M}O_2$ from SMR was observed. $S_{\text{crit}}$ represents the point at which fish transition from being an oxygen regulator to an oxygen conformer and was therefore considered as the oxygen saturation level at which fish could no longer maintain SMR. $S_{\text{crit}}$ was calculated as 1) individual $S_{\text{crit}}$ and 2) overall $S_{\text{crit}}$. Individual $S_{\text{crit}}$ was resolved using the methodology of Schurman and Steffensen (1997) where individual $\dot{M}O_2$ values below SMR were used to construct a linear regression of $\dot{M}O_2$ against $O_2$ saturation (with a forced y intercept of zero). The $O_2$ saturation level at which the regression intercepted with SMR was then taken as the $S_{\text{crit}}$ level of each individual. Individual $S_{\text{crit}}$ was then averaged and compared statistically between the 5 NTU treatments. To validate individual $S_{\text{crit}}$, overall $S_{\text{crit}}$ was also calculated using the methodology of Cook and Herbert (2012). This method employed a one-way repeated measures ANOVA to test the null hypothesis that average $\dot{M}O_2$ values under declining oxygen were not significantly less than mean SMR under normoxic conditions. A post-hoc test identified $\dot{M}O_2$ values that failed this hypothesis and this data was included in a linear regression, with a forced y intercept of zero. SMR was extrapolated across the entire range of water oxygen saturation and the associated point of intercept between the two regressions was taken as overall $S_{\text{crit}}$.

Once both measures of $S_{\text{crit}}$ were resolved, fish were returned to their respective treatment tank for 2- 5 days. The maximum metabolic rate (MMR) of fish was then determined using the exhaustive chase protocol of Cook et al. (2011) (see also review by Norin and Clark, 2016) where fish were manually chased with tail taps in a circular tank to the point of exhaustion for 5 min. Fish were then transferred immediately to the respirometer and MMR was taken as the highest of at least three $\dot{M}O_2$ values. Aerobic metabolic scope (AMS) was calculated as the difference between MMR and SMR (Schurmann and Steffensen, 1997).
Following MMR and AMS calculation, fish were removed from the chamber and euthanized using the iki-jime technique. The first left gill arch of each fish was removed immediately for histological analysis (see below) and fish were measured for length and weighed.

Fish weight varied 26.2-132 g so, to account for any potential body mass scaling effects in the data, all $\dot{M}O_2$ values were standardised (corrected) to that of a 70 g fish using the following equation:

$$
\dot{M}O_2(70g) = \dot{M}O_2(meas) \left( \frac{w}{w(70g)} \right)^{(1-A)}
$$

Where $\dot{M}O_2$ (70g) is the $\dot{M}O_2$ for a fish with the standardised (corrected) new weight of 70 g, $\dot{M}O_2$ (meas) is the measured $\dot{M}O_2$, $w$ is the weight of the fish, $w(70g)$ is the standardised body weight of fish set to 70 g and $A$ is the weight exponent describing the relationship between metabolic rate and body weight. A mass scaling exponent of $A = 0.8$ was employed (Skov et al., 2011, Skov et al., 2015).

### 2.2.3 Histological analysis of gill tissues

Immediately following euthanasia the first left gill arch of each fish was dissected out and fixed in Bouin’s solution for 48 hours, then transferred to 70 % ethanol. Gill sectioning and histological preparation was carried out by Gribble Veterinary Pathology services (Mt Wellington, Auckland) where tissues were dehydrated through a series of graded ethanol concentrations (70, 95 and 100 %) and embedded in a mould to form tissue blocks. Microtomy was then performed at 3µm to produce gill sections. These sections were mounted on glass slides and stained with haematoxylin and eosin (H & E). Samples were then examined under microscope (Leica DMRE, Wetzlar, Germany) equipped with a colour video camera (Leica DC500, Heerbrugg, Switzerland). One image of each of the samples was taken at a
magnification of 2.5 x to measure the density of secondary lamellae (termed lamellae herein). 3-5 filament sections were also randomly selected from these images to account for variability across the gill and photographed at 40 x magnification. At this magnification 4 lamellae (2 on each side of the filament) were randomly selected to measure epithelial thickness, epithelial lifting, oxygen diffusion distance and basal hyperplasia. These analyses were carried out using SigmaScan Pro 5 based on measurements used by Au et al. (2004) and Hess et al. (2015) and were carried out blind with respect to treatments.

At 2.5 x magnification, lamellae density was calculated as the number of lamellae per µm of filament length. For each fish three full filaments were randomly selected, after which the number of lamellae was counted and divided by the length of the filament. Lamellae density was therefore based on an average of 3 data values from each fish in each treatment group. At 40 x magnification, more replicated measures of lamellae condition were calculated for each fish as follows. A lamella comprises of a number of different tissues, including the outer epithelial tissue region (ET) and the inner pillar system (PS). Between these two tissues there can be non-tissue space (NT). These tissues added together make up the lamellae area (L). The area of these different tissues were measured in SigmaScan using the trace measurement mode. Parameters calculated from these measurements are as follows: 1) Thickness of the epithelium; epithelial thickness was calculated as the percentage area of epithelial tissue (ET/L). 2) Epithelial lifting; calculated as the percentage area of non-tissue space (NT/L) (however it should be noted that not all measures revealed signs of epithelial lifting - see statistical analysis section below). 3) Oxygen diffusion distance; the area of the pillar system (PS) was subtracted from the area of the functional lamella (L) then divided twice by the length of the functional lamella to obtain the oxygen diffusion distance. 4) Basal hyperplasia; thickness of the filament (µm) measured from the epithelial edge of the filament to the filament mid-line. This is a measure of the addition of cells on the filament at the base of lamellae which may thicken
progressively until two lamellae are completely fused (Wong et al., 2013). For epithelial thickness, epithelial lifting, oxygen diffusion distance and basal hyperplasia, the following number of replicated measures were made for the following number of fish: N = 9 fish and 100 lamellae for the control group, N = 8 fish and 78 lamellae for the 20 NTU treatment group, N = 8 fish and 74 lamellae for the 40 NTU treatment group, N = 9 fish and 119 lamellae for the 60 NTU treatment group and N = 10 fish and 114 lamellae for the 80 NTU treatment group.

2.2.4 Statistical analysis

Individual one-way ANOVAs were employed to examine the existence of treatment differences between the 5 turbidity groups in terms of mean SMR, MMR, AMS, $S_{\text{crit}}$, SGR and all gill morphometric measures (i.e. mean lamella density, basal hyperplasia, epithelial thickness, epithelial lifting and oxygen diffusion distance). Where the effect of turbidity treatment was found to be positive, specific pairwise comparisons between the 5 treatments was carried out using a Holm Sidak post-hoc test. All analyses were performed in Sigmaplot v. 12.5 but the parametric 1-way ANOVA was only carried out if the assumptions of normality and homoscedasticity were satisfied. To satisfy this requirement, some data were log-transformed but, where assumptions could not be satisfied, a Kruskal-Wallis one-way ANOVA on ranks was performed with a Dunn’s post-hoc test for specific pairwise comparisons. Statistical comparisons could not be attempted on data showing the total average of epithelial lifting between treatments because epithelial lifting was not evident in all samples (i.e. zeros were present in the data). Therefore, to show the true extent of epithelial lifting when present, data relating to no epithelial lifting was excluded and the remaining data was re-plotted and subject to statistical testing as above. Significance was accepted at P < 0.05 in all cases.
2.3 RESULTS

2.3.1 Gill analysis

Lamellae density. Turbidity exerted a highly significant effect on the density of lamellae on the gills (1 way ANOVA. $F = 7.66$, $df = 4$, $P < 0.01$). Control group lamellae were mostly intact and of equal length (Fig. 2.3a) but lamellae were either missing, fused, shorter or of non-uniform length at 80 NTU (Fig. 2.3b). Post hoc tests confirmed this trend because lamellae density decreased with increasing turbidity, with significant differences observed at 40, 60, 80 NTU with respect to the control < 10 NTU treatment ($P < 0.05$) (Fig. 2.4a). Basal hyperplasia. There was a highly significant turbidity treatment difference in terms of basal hyperplasia (Kruskall-Wallis ANOVA. $H = 14.27$, $df = 4$, $P < 0.01$), which increased with increasing turbidity, with significant differences observed between at 60 and 80 NTU with respect to the control (< 10 NTU) ($P < 0.05$) (Fig. 2.4b). Epithelial lifting. Of the gill samples that showed epithelial lifting, turbidity was found to have a highly significant effect on the extent of lifting (1 way ANOVA. $F = 5.62$, $df = 4$, $P < 0.01$) as lifting of the epithelium was most obvious in the high turbidity treatments (Fig. 2.3d) (Fig. 2.4c). Post hoc tests confirmed this trend with a significant difference between 80 NTU and the control (< 10 NTU) ($P < 0.05$) (Fig. 2.4d). Thickness of epithelium. Turbidity was found to have no effect on epithelial thickness (1 way ANOVA. $F = 0.42$, $df = 4$, $P > 0.05$) (Fig. 2.4e). Oxygen diffusion distance. There were highly significant turbidity treatment differences in terms of oxygen diffusion distance (Kruskall-Wallis ANOVA, $H = 20.64$, $df = 4$, $P < 0.01$). Oxygen diffusion distance progressively increased with increasing turbidity treatment with significant increases observed at 60 and 80 NTU with respect to the control ($P < 0.05$) (Fig. 2.4f).
Figure 2.3 Light micrographs of gills from *P. auratus*. a) Control lamellae at 2.5 x mag. Filaments are relatively uniform in length, lamellae are dense and mostly intact. b) Lamellae exposed to 80 NTU at 2.5 x mag. Filaments are not of uniform length and many lamellae are missing or fused. c) Control lamellae at 40 x mag showing epithelial tissue region (ET), the pillar system (PS). Non tissue space within the epithelial tissue is not conspicuous. d) Lamellae exposed to 80 NTU at 40 x mag. Non-tissue space (NT) underneath the epithelial region is obvious, leading to epithelial lifting.
Figure 2.4 Morphometric measures (mean ± 95 % CI) of *P. auratus* secondary lamellae exposed to 5 turbidity treatments (< 10, 20, 40, 60 and 80 NTU). a) Secondary lamellae density expressed as the number of lamellae per µm length of gill filament. b) Hyperplasia at the base of lamellae. c) Average epithelial lifting (including no lifting) represented by the percentage of the lamella area (L) occupied by non-tissue space (NT). d) The extent of epithelial lifting where present represented by the percentage of the lamella area (L) occupied by non-tissue space (NT). Data not showing epithelial lifting is excluded. e) Epithelium thickness represented by the percentage of the lamella area (L) occupied by epithelial tissue (ET). f) Oxygen diffusion distance. Bars with different letters are significantly different (P < 0.05).
2.3.2 Respirometry

Turbidity had no effect on SMR (F = 1.28, df = 4, P > 0.05), MMR (F = 0.78, df = 4, P > 0.05), AMS (F = 1.38, df = 4, P > 0.05) or $S_{crit}$ (F = 1.06, df = 4, P > 0.05). There was therefore no statistically significant difference between treatment groups for any of the metabolic parameters measured (Fig. 2.5). Overall $S_{crit}$ measures were generally within the measurable range of individual $S_{crit}$ measures at each NTU level (Fig. 2.5), except perhaps at 20 NTU where overall $S_{crit}$ (26.6 % O$_2$ sat) appeared slightly lower than the rest of the 20 NTU treatments (27.4 - 31.5 % O$_2$ sat).
(See legend overleaf)
**Figure 2.5** The whole animal O\(_2\) consumption rates (\(\dot{M}O_2\)) of *P. auratus* exposed to the 5 turbidity treatments (< 10, 20, 40, 60 and 80 NTU) at 18 °C. N = 14, 9, 9, 10, 11 for each treatment group respectively. The closed round symbol identifies SMR under normoxic oxygen saturation. Mean SMR was extrapolated across the range of oxygen saturation levels investigated (solid horizontal line) with associated error values indicated (dotted horizontal lines). The square closed symbol indicates mean MMR values under normoxic oxygen saturation. Open circular symbols indicate mean \(\dot{M}O_2\) values during the progressive reduction in water oxygen saturation. \(\dot{M}O_2\) values that were found to be significantly below SMR are denoted with an asterisk. A sloping regression is plotted through these values with a forced y intercept of 0. The point of intercept between this regression line and the extrapolated mean value of SMR indicates the point of overall S\(_{\text{crit}}\) and is denoted by a thin vertical black line. Mean individual S\(_{\text{crit}}\) is also shown with a solid vertical red line and 95 % confidence limits as vertical dotted red lines. See the methods section for more detail regarding S\(_{\text{crit}}\) calculations. All symbols and error bars represent mean values ± 95 % confidence intervals.
2.3.3 Growth

Turbidity had a highly significant effect on fish SGR over 30 days of NTU exposure (Kruskal-Wallis ANOVA, $H = 25.76$, df = 4, $P < 0.01$). Controls generally maintained their weight but there was a significant loss of weight observed at 80, 60 and 40 NTU with respect to the control ($< 10$ NTU) ($P < 0.05$) (Fig. 2.6).

**Figure 2.6** The mean somatic growth rate (SGR) of *P. auratus* exposed to the 5 turbidity treatments over 30 days. Data is presented as mean ± 95% CI. An asterisk indicates a significant difference from the control ($P < 0.05$).
2.4 DISCUSSION

There are several reports of fish suffering gill damage as a result of suspended sediment exposure (Hess et al., 2015, Lowe et al., 2015, Wong et al., 2013) and these fish also appear to show slower rates of growth or longer rates of development and even mortality (Lowe et al., 2015, Wenger et al., 2012, Wong et al., 2013). Authors of these studies have logically speculated that oxygen transfer across damaged gills must be sufficiently impaired and that fish experience a long term cost with respect to individual fitness performance. However, being the first to study the aerobic physiology of fish from turbid waters, the current study does not support this hypothesis. Juvenile *P. auratus* that were exposed to increasingly turbid conditions did indeed show significant gill structural change and weight loss as seen in previous studies (Figs. 2.3, 2.4 & 2.6) (Lowe et al., 2015), but there was no measurable change in any measure of oxygen uptake under well oxygenated conditions (normoxia) and fish could even maintain the same rate of oxygen uptake during a low oxygen challenge (hypoxia) test (Fig. 2.5). Therefore, contrary to logical expectations, the fitness deficits of fish exposed to suspended sediment may not be due to impaired oxygen transfer, at least not in *P. auratus* that inhabit turbid waters around the coast of New Zealand.

2.4.1 Gill structural response to turbid waters

 Significant gill structural change in response to increasingly turbid waters was evident in *P. auratus* and was characterised not only by a measurable decrease in gas exchange surface area but also an increase in gas diffusion distance at the level of the secondary lamellae. Loss of secondary lamellae is considered to be the direct net effect of sediment particle abrasion (Lake and Hinch, 1999) and individual *P. auratus* showed a decrease in lamellae density on the first gill arch with increasing levels of turbidity (Fig. 2.4a). If taken as a true representation of
change across the whole gill, this observation alone should indicate that the oxygen uptake potential of *P. auratus* in turbid treatments is limited by a significant reduction in functional gill surface area. Similarly, the observed increase in basal hyperplasia with increasing turbidity (Fig. 2.4b) (which is thought to precede complete lamellae fusion) would also be expected to further reduce the gill surface area for oxygen exchange.

*P. auratus* exposed to increasingly high NTU treatments showed a progressive increase in oxygen diffusion distance across the secondary lamellae that was driven entirely by epithelial lifting (Fig. 2.4c & d) rather than epithelial thickening (Fig. 2.4e). This is presumably a response to protect the inner pillar cell system from the abrasive action of suspended sediment and to prevent irritants from diffusing into the blood stream (Mallatt, 1985). Au *et al.* (2004) also found evidence of epithelial lifting in juvenile green grouper (*Epinephelus coioides*) exposed to ≥ 200 mg l⁻¹ of suspended sediment compared to the controls. Similarly, Wong *et al.* (2013) documented evidence of epithelial lifting and hyperplasia from the base of the lamellae exposed to suspended sediment. Another related study by Hess *et al.* (2015) showed that tropical clownfish larvae exposed to suspended sediment experienced a 56 % increase in oxygen diffusion distance but this difference was the result of hyperplasia of the epithelial tissue (Hess *et al.*., 2015). Epithelial hyperplasia was also observed with increasing turbidity in a study by (Lowe *et al.*, 2015). Although these studies do not necessarily assess the same parameters nor obtain the same results as the current study, one consistent feature is that they all show structural changes synonymous with an increase in the diffusion distance of the lamellae. Not surprisingly some authors speculated that this may reduce the efficiency of oxygen uptake across the gills, which could result in a reduction of oxygen transport to the organs, causing respiratory stress (Au *et al.*, 2004, Hess *et al.*, 2015, Lowe *et al.*, 2015, Wong *et al.*, 2013). In support of such speculation, studies by Bindon and colleagues (1994a and 1995b) also suggest that structural change brought about by chloride cell proliferation on the
secondary lamellae can negatively impact blood oxygen and carbon dioxide saturation. Chloride cells play a vital role in osmoregulation, however proliferation of chloride cells have been demonstrated to benefit ionic regulation at the expense of arterial blood oxygenation (Bindon et al., 1994a, Bindon et al., 1994b). Furthermore, chloride cell density has been shown to increase with turbidity (Au et al., 2004, Wong et al., 2013), further reinforcing the view that changes in gill structure as a result of turbidity exposure may impair the oxygenation of blood (Bindon et al., 1994a, Bindon et al., 1994b).

Furthermore, for effective gaseous exchange there is the expectation that the epithelial diffusion distance between inhalant water and the pillar cell system should be as minimal as possible (Evans et al., 2005). Oxygen diffusion distances in fish are reported to range from 0.7–10 µm (Evans et al., 2005) and this appears to correlate directly with the aerobic athleticism of different fish species. Therefore, on the basis that membrane thickness is optimised to satisfy the aerobic requirements of a given species, the notion that a turbidity-related increase in oxygen diffusion distance would impair oxygen uptake requirements does not seem unreasonable.

### 2.4.2 Rates of oxygen consumption in response to turbid water

The current study set out to resolve whether gill structural change via turbidity exposure would reduce the capacity for oxygen transfer but, despite evidence of major gill restructuring, no significant change in oxygen uptake was found with increasing turbidity. All metabolic measures of SMR, MMR and AMS as well as $S_{crit}$ breakpoints were comparable between the five turbidity treatments (including the control) with no marked differences between any of the groups. These results therefore oppose the speculative discussion of several studies (e.g. Hess et al., 2015, Lowe et al., 2015) where prolonged development and slow growth was deemed the result of respiratory stress brought about by suspended sediment and structural gill change.
If a situation occurred where the gills of *P. auratus* were structurally damaged and did indeed impair oxygen uptake capacity, it is foreseeable that a range of physiological adjustments could feasibly compensate for reduced oxygen transference across the gills so that basal and/or routine metabolic function (SMR) was maintained. For example, Dussault *et al.* (2001) discovered that structural changes to the gills of rainbow trout as a result of aluminium exposure led to an increase in blood hematocrit (Hct) and blood haemoglobin concentration (Hb). In more severely affected fish, the raise in Hct and Hb was also associated with an increase in heart rate and stroke volume (Dussault *et al.*, 2001). There is less physiological information available for fish exposed to turbidity but, an increase in the rate of gill ventilation has been observed in *P. auratus*, although how this contributed to oxygen uptake was not ascertained in that study (Lowe *et al.*, 2015). In another study of interest, Gold *et al.* (2015) showed that an increase in cardiac output was sufficient to maintain routine oxygen consumption in anaemic rainbow trout that had reduced aerobic capacity (Gold *et al.*, 2015).

Whilst SMR might be held constant through a plethora of compensatory mechanisms, it is far harder to understand how MMR (as an indicator of maximum aerobic performance) could be held steady with structurally modified gills, as seen in the current study. There is simply no evidence that turbidity affects the oxygen extraction capacity or efficiency of this species. Whilst contradictory to the main hypothesis of the study and that of Hess *et al.* (2015) and Lowe *et al.* (2015), high NTU treatments maintained MMR and $S_{\text{crit}}$ with apparently damaged (or at least highly modified) gills and serves to challenge the concept of symmorphosis. Symmorphosis states that biological design is optimised and that structure matches functional requirements with no excess provisions (Taylor and Weibel, 1981) but the results of the current study do not support this theory as the oxygen uptake rates of higher NTU treatments were exactly the same as the $< 10$NTU controls, despite the presence of fewer secondary lamellae with increased oxygen diffusion distance. It is therefore concluded that $<$
10NTU controls do not show an optimised design but instead have gills furnished with secondary lamellae and thin epithelia, that are potentially in excess of functional requirements. A number of other studies also present evidence of “excessive construction” that refute the concept of symmorphosis (Chappell et al., 2007, Garland and Huey, 1987) but, as argued by Randall and Brauner (1991), “structures are designed to satisfy functional requirements for operation over a wide range of conditions, rather than optimally for a given set of conditions”. Therefore, *P. auratus* may possibly maintain reserve gill capacity under non-turbid conditions as an evolutionary safeguard against diminished respiratory performance at times of environmental stress (e.g. increased turbidity). The loss of lamellae surface area may not therefore result in the loss of respiratory performance. Studies on the crucian carp, *Carassius auratus*, reinforces this view because this species is shown to maintain routine metabolic function under well oxygenated conditions with simple gills that lack protruding lamellae (Sollid et al., 2003).

### 2.4.3 The fitness consequence of turbidity exposure

With respect to the growth of fish in turbid waters this study is consistent with the observations of others because increasing turbidity was shown to impair fish productivity, presumably as an indirect fitness cost. Indeed, fish in all turbidity treatments except the < 10 NTU control group lost significant weight and the greatest weight loss occurred in the highest turbidity group. The expectation that all fish would show positive gains in biomass is probably unrealistic for an experiment of this nature due to i) the turbulent nature of water in the tanks that held turbidity treatments constant, ii) the regular disturbance and handling of fish, and iii) the use of 18.0 °C water, which is a few degrees below the optimal growing temperature of *P. auratus* (Fielder et al., 2002, Francis, 1997). As such, it is encouraging that fish in the < 10NTU treatment
maintained their weight across the 30 day experiment, especially as the control turbidity treatment in the study of Lowe et al., (2015) lost significant weight.

2.4.4 Conclusion

In conclusion, the most important question posed by the speculative discussion of Hess et al., (2015) and Lowe et al., (2015) was whether the fitness consequence of growth loss is the direct result of impaired oxygen transfer across the gills? The current study does not however provide any evidence to support this claim because the respiratory performance of P. auratus was not impaired at higher NTU despite evidence of significantly greater weight loss and gill structural change. It is therefore concluded that “respiratory stress” is not the cause of the growth deficit in P. auratus and that some other factor must be involved. Chapter 3 goes part way in answering this question by examining whether reduced growth is simply due to the loss of visual feeding performance in turbid waters (Johansen and Jones, 2013, Meager and Batty, 2007). However, future research may also wish to test whether some other physiological perturbation is involved and this is a topic of discussion in Chapter 4 (subsection 4.3.1).
3 EFFECTS OF TURBIDITY ON THE FEEDING BEHAVIOUR OF JUVENILE SNAPPER (*PAGRUS AURATUS*)

3.1 INTRODUCTION

Despite many environmental constraints, fish depend largely on vision as a main source of sensory information to identify and capture prey (Fielder *et al.*, 2002, Guthrie, 1986, Johansen and Jones, 2013, Utne-Palm, 2002) and therefore depend solely on ambient light to see their prey (Higham *et al.*, 2015). Suspended sediment in the water column reduces water clarity and can have a drastic impact on the visual range of predatory fish species and their ability to see prey (Higham, *et al.*, 2015). A reduction in light penetration as a result of increases in suspended sediment has been shown to affect visual feeders directly through changes in food acquisition as outlined in Chapter 1.

Suspended sediments have been shown to alter feeding behaviour in both freshwater and marine fish species through changes in prey capture success (Berg and Northcote, 1985, Johansen and Jones, 2013), reaction distance (Berg and Northcote, 1985), attack rate (Johansen and Jones, 2013), foraging rate (Johansen and Jones, 2013, Rowe and Dean, 1998), reaction time (Wenger *et al.*, 2012) and number of prey consumed (Berg and Northcote, 1985, Hasenbein *et al.*, 2013, Hazelton and Grossman, 2009, Lowe *et al.*, 2015, Wenger *et al.*, 2012). However, of ecological relevance is the fact that a reduction in food acquisition also appears to be associated with a decrease in fish fitness performance (Lowe *et al.*, 2015, Morrison *et al.*, 2009, Wenger *et al.*, 2012, Wilber and Clarke, 2001), with many studies reporting reductions in growth, weight loss or delayed development with increasing turbidity (Engstrom-Ost and Mattila, 2008, Sutherland and Meyer, 2007, Wenger *et al.*, 2014). For example, pike larvae in turbid waters showed reductions in feeding activity that appeared to be associated with weight
loss during the exposure period (Engstrom-Ost and Mattila, 2008). Similarly Wenger, et al. (2012) found that turbidity had a strong negative effect on food acquisition of planktivourous damselfish, which was accompanied by a significant reduction in growth and body condition. Suspended sediment loads therefore appear to alter fish foraging patterns and success through reductions in visual acuity and have the potential to impact on fish body condition and growth. Although some marine species such as juvenile chinook salmon (Gregory and Northcote, 1993) actually show positive changes in feeding ability with increases in turbidity (Gregory and Northcote, 1993, Morrison et al., 2009), this is not expected to be the case in the current study as previous similar work on marine species did not show positive changes in feeding behaviour with turbidity (Johansen and Jones, 2013, Lowe et al., 2015).

Chapter 2 of this thesis describes how juvenile P. auratus in turbid waters lost significant levels of weight over 30 days yet the weight of control fish did not change. This confirms that long-term turbidity exposure is somewhat detrimental to the growth productivity and fitness of juvenile P. auratus. It was originally hypothesised that this change in growth rate was an indirect result of altered gill structure, which was expected to impair oxygen uptake across the gills and limit growth. Significant changes in gill structure were indeed apparent with progressive increases in turbidity and was characterised by a measurable decrease in gas exchange surface area and an increase in gas diffusion distance at the level of the secondary lamellae (Chapter 2). However, contrary to expectations, the results showed no measurable difference in any oxygen consumption measures between the treatment groups, rejecting the hypothesis that altered gill structure impaired oxygen uptake across the gills and thus limited growth productivity and fitness. On one hand this suggests that P. auratus may in fact be more resilient to turbidity stress than previously thought, which may have significance for P. auratus in terms of individual and population health and survival in the face of a rapidly changing marine environment. However, fish lost significant weight but the mechanism that underlies
this observed growth deficit with increasing turbidity treatment is yet to be determined. One of the mechanisms suggested in Chapter 2 for reduced growth is simply the loss of visual feeding performance in turbid waters as demonstrated previously in a number of studies (e.g. Wenger et al., 2012). As such, this study aims to assess visual feeding performance as a possible mechanism for reduced growth under turbid water conditions.

Although the feeding behaviour of snapper under turbid conditions has previously been investigated (Lowe et al., 2015), this study aims to provide a more robust and in-depth examination of feeding behaviour of individual fish using the same turbidity levels as the experiments conducted in Chapter 2. Lowe et al. (2015) examined feeding rate in *P. auratus* under still water conditions so did not examine any behavioural feeding performance parameters under ecologically relevant flow conditions and was unable to separate visual from non-visual feeding cues. Although Lowe et al. (2015) witnessed differences in the proportion of prey eaten with increasing turbidity, this study aims to provide a more comprehensive and detailed investigation in an attempt to more accurately assess whether, or indeed how, a reduction in feeding is the cause of poor growth in turbidity. It was therefore specifically hypothesised that turbidity will reduce the growth fitness of *P. auratus* by limiting the strike reaction distance and food capture success of this species.
3.2 METHODS

3.2.1 Fish collection and housing

Juvenile snapper (*P. auratus*, 10.1 cm ± 0.4 cm FL) were caught using a beach seine net in the Kaipara harbour, New Zealand. Fish were housed at the Leigh Marine Laboratory in 500 L flow-through holding tanks at ambient sea temperature and fed daily on diced mussels or pilchard. Fish were held for up to 8 months prior to experimentation. All holding and experimental techniques were performed under approval of The University of Auckland Animal Ethics Committee (approval number: 001451).

3.2.2 Experimental apparatus

A 38.4 L Brett-style respirometer was modified and utilised as an experimental swim flume to test the ability of *P. auratus* to detect and capture mobile prey items in a flow, under varying levels of turbidity (< 10, 20, 40, 60 and 80 NTU). The swim flume consisted of 2 parallel working chambers (32 cm x 8 cm) allowing simultaneous observations of two fish (Fig. 3.1). A bubbler was inserted outside of the working chamber to provide aeration. Flow straighteners were used to create laminar flow within the working section. The swim flume included two pieces of plastic tubing (1 cm wide) inserted into the lid ahead of the working chamber to allow feed items to be introduced (Fig. 3.1). These were at a length that allowed feed items to enter the flow near the bottom of the water column, providing continuity in the presentation of feed between the test subjects. A catch bag made of 800 µm mesh was used to collect any feed items not caught by the test subject and was placed at end of working section. Any actions of the observer (i.e. inputting feed items) was obstructed from view by the fish using back plastic and the entire apparatus was shielded from outside stimuli using black plastic sheeting. A colour
CCD video camera (KCP-VBN190PH(H-RES)) was mounted beside the working section and was set up to record the feeding experiments via a link to a computer and a hard drive. A red light was used to backlight the working section. A number of slits were created in the plastic overhead the apparatus to allow some natural light (7.0 lux) to enter which provided illumination for the fish to feed.

Each experimental level of turbidity was created by adding estuarine sediment as a slurry as described in Chapter 2. The amount of sediment needed to create the respective turbidity levels was resolved prior to experimentation and was determined using a TSS portable handheld measurement instrument (HACH, Germany). A flow rate of 0.2 m/s within the closed system swim flume was identified in the trials as the velocity at which fish could swim for 24 hours and show no signs of stress (i.e. still feed) and at which turbidity could be maintained at each level for the duration of the experiment. At this flow rate, sediment was shown to drop an average of 14 % over this time period. Therefore, when adding sediment, the starting turbidity was always slightly higher than the target level so that the target level was reached at approximately the midpoint of the experiment.
Figure 3.1 Diagrammatic representation of swim flume set up used to measure feeding behaviour parameters for *P. auratus*. Blue arrows represent direction of water flow.
3.2.3 Experimental protocol

10 fish were randomly assigned to each turbidity level (< 10, 20, 40, 60 or 80 NTU) and foraging performance was examined at a pre-determined flow rate (0.2 m/s). At the beginning of each experiment, the flow tunnel was cleaned and filled with temperature controlled seawater (18 °C). This temperature (18 °C ± 0.6 °C) was able to be maintained throughout the duration of the experiment without manipulation. Fish were unfed for 48 h prior to experimentation to ensure maximum food consumption, then placed in the working section and left to acclimatise for at least 4 hours at a flow rate of 0.01 m/s. Experiments were commenced only if fish had settled into a continuous swimming rhythm. Following acclimation, flow rate was progressively increased over 15 minutes until a rate of 0.2 m/s was reached. Fish were allowed to acclimate to this flow rate for a further 15 min. Mosquito larvae (*Opifex fuscus*) were used as the mobile feed item and trials in clear water showed that fish would eat > 60 mosquito larvae in 15 min at a flow rate of 0.2 m/s. Following the 15 min acclimation period to the flow rate, fish were fed a number of ‘trial’ feed items until both test subjects were feeding satisfactorily. Those that did not feed satisfactorily were disregarded. At this point, sediment was added slowly to the turbidity treatment groups until it reached the desired level. Fish were then allowed to acclimate for a further 5 min before the experiment was carried out.

At each level of turbidity, the start time was recorded and 15 mosquito larvae were added individually for each fish every two minutes, during which the feeding behaviour of the test subject was recorded. Movement of the fish towards the feed items and any feeding action at this point was noted. At the conclusion of the experiment, the feed catcher at the base of the chamber was removed and any uneaten feed items were counted. This was used to calculate attack success, measured as the number of prey items caught successfully (i.e. feed delivered minus the feed captured in mesh bag). Using the video footage, a number of further parameters were measured: foraging bites were counted as in Johansen and Jones (2013) where foraging
Chapter 3

bites were determined as a clear bite (opening and closing of the mouth), generally accompanied by a distinct manoeuvre toward a particular point in the water column. Reverse foraging bites were identified as foraging bites that occurred when the point of prey capture was behind the starting point, (i.e. the test subject only saw the feed item as it went past rather than observing it and moving toward it from a distance). Attack distance was calculated as the distance from the point of origin to where a foraging bite occurred. Attack distance was calculated using Sigma Scan from still video sequences.

3.2.4 Statistical analysis

Individual one-way ANOVA tests were utilized to examine the existence of treatment differences between the 5 turbidity treatment groups in terms of feeding behaviour (mean attack success, foraging bites, attack distance and reverse attacks). Where the effect of turbidity was positive, specific pairwise comparisons between the 5 treatment groups were carried out using a Holm Sidak post-hoc test. All analyses were performed in Sigmaplot v. 12.5 but the parametric 1-way ANOVA was only carried out if the assumptions of normality and homoscedasticity were satisfied. To satisfy this requirement, log-transformations were attempted but, where assumptions could not be satisfied, a Kruskal-Wallis one-way ANOVA on ranks was performed with a Dunn’s post-hoc test for specific pairwise comparisons. To ensure results were not being skewed by other parameters such as time of day and body size, individual one-way ANOVA tests were utilized to examine the existence of differences in feeding behaviour parameters at different times of day (AM vs PM). Similarly, an individual Pearson Correlation test was carried out to examine the effect of body size on feeding behaviour. Significance was accepted at P < 0.05 in all cases.
3.3 RESULTS

Attack success. Increasing turbidity exerted a highly significant negative effect on the ability of fish to capture their prey (Kruskal-Wallis One-Way ANOVA. $H = 18.09$, $df = 4$, $P < 0.01$), with significant differences observed at 80 NTU with respect to the control < 10 NTU treatment ($P < 0.05$) (Fig 3.2a). Foraging bites. Increasing turbidity also exerted a highly significant negative effect on the number of foraging bites (Kruskal-Wallis One-Way ANOVA. $H = 23.42$, $df = 4$, $P < 0.01$), with significant reductions in bites observed at 80 NTU with respect to the control < 10 NTU treatment ($P < 0.05$) (Fig. 3.2b). Reverse foraging bites. There was a highly significant turbidity treatment difference in terms of the frequency of reverse foraging bites (Kruskal-Wallis One-Way ANOVA. $H = 24.6$, $df = 4$, $P < 0.01$) with significant increases in reverse attacks at 60 and 80 NTU with respect to the control < 10 NTU treatment ($P < 0.05$) (Fig. 3.2c). Attack distance. There were highly significant turbidity treatment differences in term of attack distance (1-way ANOVA. $F = 31.53$, $df = 4$, $P < 0.01$). Attack distance progressively decreased with increasing turbidity treatment with significant differences observed at all turbidity treatment levels (20, 40, 60 and 80 NTU) with respect to the control < 10 NTU ($P < 0.001$) (Fig. 3.2d). Results showed no difference in feeding behaviour parameters (attack success, foraging bites, attack distance) at the different times of day the experiment was run (AM versus PM, Mann-Whitney Rank Sum Test, $P > 0.05$) and no correlation between body size and feeding behaviour parameters (attack success, foraging bites, and attack distance) (Pearson correlation, $P < 0.05$) eliminating any effects of these factors.
Figure 3.2 Feeding behaviour parameters (mean ± 95% CI) of juvenile *P. auratus* during feeding behaviour experiments in a swim flume under increasing levels of turbidity (< 10, 20, 40, 60 and 80 NTU). a) Attack success measured as the total number of prey items (*Opifex fuscus*) caught successfully out of 15. b) The total number of foraging bites during feed presentation. c) Reverse foraging bites measured as a proportion of the total number of bites during feed presentation. d) Attack distance measured in cm as from the point of origin to the point where a foraging bite occurred during distinct manoeuvres towards a particular point in the water column. Bars with different letters are significantly different (P < 0.05).
3.4 DISCUSSION

The growth of *P. auratus* was severely impacted as a result of suspended sediment exposure (Chapter 2). However, altered gill structure as a result of turbidity exposure had no impact on the ability of fish to take up oxygen across the gills (Chapter 2) and therefore questions the mechanism by which these fish lost weight. There are several reports of fish experiencing a decreased ability to forage during turbidity exposure (Berg and Northcote, 1985, Johansen and Jones, 2013, Lowe et al., 2015, Wenger et al., 2012,) and some studies have reported a link between a reduction in feeding ability and decreased fish growth (Lowe et al., 2015, Wenger et al., 2012, Wilber and Clarke, 2001). This study supports the idea that turbid waters have the potential to limit the visual performance of fish and affect their ability to forage. Juvenile *P. auratus* that were exposed to increasingly turbid conditions did indeed show significant changes in feeding behaviour as seen in previous studies (Johansen and Jones, 2013, Lowe et al., 2015, Wenger et al., 2012). Therefore, this study provides evidence to support the hypothesis that reductions in growth performance (Chapter 2) may be at least in part due to alterations in prey acquisition rather than deficits in oxygen consumption.

As fish rely on vision to identify prey (Utne-Palm, 2002) it is not surprising that many studies demonstrate a decrease in foraging ability when exposed to turbid conditions, including increases in reactive distance and decreases in attack success (Johansen and Jones, 2013, Utne-Palm, 1999, Wenger et al., 2012). The results from this study, where the foraging ability of juvenile snapper on *O. fuscus* was shown to decline with increasing turbidity, are consistent with prior research (Johansen and Jones, 2013, Lowe et al., 2015, Utne-Palm, 1999, Wenger et al., 2012). Specifically, an increase in reactive distance was seen at all turbidity levels and feeding success was impacted at the highest NTU. Although this study reports similar findings to the turbid feeding behaviour of tropical fish species (Johansen and Jones, 2013, Wenger et
al., 2012), the threshold level of response is less sensitive for *P. auratus* in temperate waters. For example, our study reports a reduction in attack success at 80 NTU. In contrast, Johansen and Jones (2013) report that attack success was reduced at only 8 NTU for their coral reef fish species that normally reside in clear water. Similarly, the tropical fish species *A. polyacanthus* showed a significant decrease in food acquisition at 30 NTU (Wenger *et al.*, 2012). These disparities in thresholds are to be expected and may simply reflect individual species-specific tolerance which may in turn be related to habitat. For example, it is logical that coral reef fish would more be particularly sensitive to increased turbidity due to the natural clarity of the waters in which they reside and are adapted to (Johansen and Jones, 2013). To highlight this, Johansen and Jones (2013) found that feeding behaviour response of all species of coral reef fish investigated was proportional to levels they may naturally encounter in the field. For example, inshore species of their study naturally encounter higher levels of turbidity and thus showed no effect at 4 NTU, whereas mid to outer shelf species that experience clear water all showed significant reductions in attack success at this level (Johansen and Jones, 2013). At turbidity levels above those normally encountered in the field, all species showed major reductions in attack success (Johansen and Jones, 2013). In this sense, the thresholds where effects of turbidity may occur appear to depend on what that species may naturally encounter in their environment and thus this must be considered when comparing the current set of results to other species.

The current study is compatible with the results of Lowe *et al.*, (2015) because feeding behaviour was studied under similar NTU levels and differences were witnessed in the capture success of mobile prey items. However, the current study does provide an extra level of detail and is associated with very important detail in the methodology, namely the ability to separate visually orientated feeding from non-visual feeding responses, as well as disconnect individual feeding from group feeding. For example, the study of Lowe *et al.*, (2015) simply placed feed
items into low flow turbid tanks with *P. auratus* and allowed their fish to forage over time. Realistically, fish in their study were probably able to use visual and/or non-visual (chemical) cues to find prey so could not entirely test the effect of turbidity on visual feeding behaviour alone. On the other hand, by requiring fish to forage rapidly in a swim flume in the current study, our methodology was more effective in eliminating the influence of chemical feeding cues in the location of prey. In addition, the methodology of Lowe *et al.*, (2015) saw feeding success calculated using 3 fish per treatment tank, which may have affected the results through the influence of conspecifics. Our methodology was perhaps more effective in assessing the effects of turbidity alone on feeding behaviour of *P. auratus* through the elimination of the influence of conspecifics, reducing variable factors that may affect feeding behaviour. As such, due to differences in methodology, a difference in threshold sensitivities between the two studies with regards to feeding/capture success was expected. Threshold differences were in fact apparent, with a significant decrease in capture success at 80 NTU in the current study compared to a lower threshold of 40 NTU in the study of Lowe *et al.*, (2015), however, these differences cannot be explained by differences in methodology described above.

Comparing the feeding behaviour response of *P. auratus* under laboratory conditions is insightful, but care needs to be taken when extrapolating these results to fish in the wild because several factors have not been considered. Due to the design of the swim flume, feed items passed in close proximity to the test subject for the majority of the time, which increased the chance of fish capturing feed items (i.e. the distance they had to move to capture the feed was generally low). In the wild, however, fish are unlikely to be presented with as many ‘close range’ feeding opportunities and thus may either a) need to expend more energy searching for prey, or b) consume less food items than they normally would under non-turbid conditions. Therefore, in a realistic situation, although attack success was only significantly lower at 80 NTU in this study, this would possibly become more significant at lower NTU levels if
proximity to prey was decreased. Future research may wish to explore this possibility but it is also important to consider differences in the energetics of feeding for fish in these experiments and fish in the wild. Fish in the wild may be metabolically burdened because patchy food distribution and random presentations would increase the energetic cost of fish finding food under turbid conditions. For example, under limited food availability, individuals of the marine damselfish Pomacentrus chrysurus show declines in physical condition and expose themselves to greater risks of predation in an attempt to find food that satisfies their energetic requirements (Lienart et al., 2014). Furthermore, the cost of swimming is a substantial component of the energy budget of a fish (Boisclair and Sirois, 1993, Ruzicka and Gallager, 2006). Therefore, any extra energy spent on foraging is likely to impose a large energetic cost on fish, using energy that would otherwise be prioritised for growth and reproduction (Buraeu et al., 2002). Fish that are unable to obtain substantial quantities of feed may also be forced to down-regulate certain physiological activities to reduce energy use (Mehner and Wieser, 1994) (e.g. decrease in cardiorespiratory activity and metabolic enzyme activity) (Collins and Anderson, 1997), which may cause reductions in swimming performance (Fu et al., 2011) further reducing the foraging potential of the given species. On the other hand, if fish are not able to rely on vision to effectively forage for feed items, this may force snapper to rely on other sensory feeding modalities, such as olfaction.

One of the aims of this study was to investigate whether or not reductions in feeding behaviour could in fact be plausible as an alternative/complementary hypothesis to the growth deficits seen during long term turbidity exposure in Chapter 2. The results of this study clearly demonstrate a reduction in feeding ability with increasing turbidity and suggest that feeding behaviour may be sufficiently altered to the point where positive fish growth is impacted. Although reduced feeding as a mechanism for weight loss was not measured directly in this study, it is commonly understood that fish must consume enough food to satisfy their energetic
requirements for maintenance, growth and reproduction (Killen, 2011). Energy input in the form of food is required in order to sustain biological function and, as such, it is assumed that energy (food) intake is positively correlated with fish fitness (Killen, 2011). Therefore, variables such as turbidity that prevent fish from acquiring sufficient food to satisfy their energy requirements, are logically thought to affect fish fitness (i.e. fish growth). Furthermore, to achieve maximum rate of energy intake through ingestion of feed items, fish must also minimise the amount of energy spent foraging (Killen, 2011) as per the basis of optimal foraging theory. Therefore, *P. auratus* under turbid conditions may either receive less energy intake through diminished food intake and/or may have to utilise more energy in foraging. Future work may need to disentangle these mechanisms but, for now, it is clear that the growth of *P. auratus* is affected by turbidity exposure, most probably through feeding limitation as opposed to impaired oxygen uptake.

In conclusion, our results demonstrate that the feeding behaviour of *P. auratus* is influenced by turbidity through direct negative impacts on visual acuity alone. This reduction in feeding ability is correlated with increasing turbidity and may affect the prey acquisition of *P. auratus* to the point of negatively impacting growth. As such, reductions in feeding with increasing turbidity may explain in part the growth deficits seen in Chapter 2 as opposed to impaired oxygen uptake. These results may be of particular significance to *P. auratus* in the wild and if turbidity is persistent, subsequent decreases in feeding could have serious consequences through reductions in growth, with potential flow on effects on fish condition, reproduction, population dynamics and survival (Killen, 2011).
4 GENERAL DISCUSSION

The results of the research presented in this thesis provide the first detailed insight into the aerobic physiology and feeding behaviour of *P. auratus* exposed to turbid waters and examines in detail changes in gill structure, growth and prey acquisition. An understanding of the mechanisms by which turbidity can influence important fish species such as *P. auratus* is critical for effective management of suspended sediment in the coastal marine environment of New Zealand, particularly in the Hauraki Gulf.

4.1 IMPACT OF TURBIDITY ON GILL STRUCTURAL CHANGE

As predicted, turbidity exposure had a significant effect on the gills of *P. auratus*, eliciting a number of structural gill changes including a reduction in lamellae density, an increase in basal hyperplasia and an increase in oxygen diffusion distance, driven primarily through lifting of the epithelial tissue (Chapter 2). These results support the original hypothesis that *P. auratus* would exhibit significant changes in gill structure with increasing turbidity exposure as seen in previous studies (Au *et al.*, 2004, Hess *et al.*, 2015, Wong *et al.*, 2013). These results are not entirely surprising because changes in gill structure such as epithelial lifting (Au *et al.*, 2004, Wong *et al.*, 2013), increased oxygen diffusion distance and epithelial hyperplasia (Hess *et al.*, 2015) have been observed in a number of species following turbidity exposure, including clownfish (Hess *et al.*, 2015) and juvenile green grouper (Au *et al.*, 2004, Wong *et al.*, 2013). Gill changes in *P. auratus* following turbidity exposure have also previously been investigated as part of a broader research topic by Lowe *et al.*, (2015) with results demonstrating dilation of the lamellae. However, for the purposes of the subsequent experiments in this study it was
imperative to confirm that *P. auratus* in the current study did indeed experience gill changes such as those seen in previous work (e.g. Lowe *et al.*, 2015). As such, a detailed investigation of structural gill change in *P. auratus* was carried out using methodology from previous papers that specifically address gill damage (Au *et al.*, 2004, Hess *et al.*, 2015). This consequently provides a more comprehensive understanding of structural gill change in *P. auratus* following turbidity exposure as a means of complementing and moving forward from previous research carried out by Lowe *et al.*, (2015).

The results of this study are consistent with the earlier findings of others showing changes in gill structure at the level of the secondary lamellae such as epithelial lifting (Au *et al.*, 2004, Hess *et al.*, 2015, Wong *et al.*, 2013). However, the thresholds at which significant gill changes occur appears to differ between studies. This may be related to the habitat of the species investigated and the background levels of turbidity that these fish may experience. For example, results from this study revealed significant changes in gill structure (specifically oxygen diffusion distance) at approximately 60 NTU. This is in contrast to other studies where significant changes in gill structure, affecting oxygen diffusion distance, occurred at approximately 200 mg/l (~ 40 NTU) for juvenile green grouper (*E. coioides*) (Au *et al.*, 2004), 500 mg/L (~ 80 NTU) for the freshwater species *Erimonax monachus* (Sutherland and Meyer, 2007) and as low as 2.5 NTU for clownfish larvae (*Amphiprion percula*) (Hess *et al.*, 2015). Although these thresholds may differ depending on the species, the key point of interest is that fish exposed to turbidity almost universally show an increase in oxygen diffusion distance across the lamellae, regardless of the species, thresholds or the methodology by which it is measured.
4.2 Impact of Turbidity on Oxygen Consumption

Fish gill epithelia effectively provide a highly efficient surface for gas exchange (Evans et al., 2005). Therefore, any increase in oxygen diffusion distance across the lamellae that occurs as a result of turbidity-related gill damage (as described above) is thought to have a significant negative effect on fish health (Hess et al., 2015, Lowe et al., 2015, Wenger et al., 2012, Wong et al., 2013). Specifically, alterations in diffusion distance are logically speculated to impact gas exchange (Hess et al., 2015, Lowe et al., 2015, Wong et al., 2013) and thus it was hypothesised that oxygen uptake efficiency of *P. auratus* would be reduced as a direct result of increased oxygen diffusion distance across the lamellae. However, the results of this thesis provide the first experimental evidence that the whole animal oxygen uptake rate of *P. auratus* is not affected by turbidity-induced gill damage. This is a surprising result that contravenes the logical speculation of others (Hess et al., 2015, Lowe et al., 2015, Wong et al., 2013) and therefore the main hypothesis of even our own study. The fact that turbidity-induced gill damage does not alter the oxygen uptake ability of *P. auratus* challenges the concept of symmorphosis. Indeed the results provide evidence suggesting *P. auratus* typically maintains structure in excess of functional requirements and does not have an optimised design for oxygen uptake under routine conditions as the theory of symmorphosis states (Taylor and Weibel, 1981). As such, this demonstrates that the oxygen uptake potential of fish gills is perhaps more resilient to environmental change than first thought, which may be of significance for *P. auratus* living in an increasingly changing environment. *P. auratus* can, after all, maintain satisfactory oxygen uptake with quite severely damaged gills.
4.3 IMPACT OF TURBIDITY ON FISH GROWTH

Although no alterations in oxygen uptake ability were observed, fish still lost significant weight from the beginning of turbidity treatment to the conclusion (Chapter 2). As other environmental factors such as temperature was kept constant throughout the experimentation period, it must be concluded that turbidity has a negative impact on the growth of *P. auratus*. This result is consistent with previous research where reductions in growth, weight loss and delayed development have been reported with increasing turbidity (Engstrom-Ost and Mattila, 2008, Lowe *et al.*, 2015, Sutherland and Meyer, 2007, Wenger *et al.*, 2012, Wenger *et al.*, 2014). For example, significant reductions in the growth of planktivorous damsel fish were observed in turbidity treatment of just 7.5 NTU (Wenger *et al.*, 2012). Similarly, the larvae of coral reef fish *A. percula* had significantly longer larval duration under turbidity treatments as low as 2.5 NTU (Wenger *et al.*, 2014). However, as oxygen consumption was unaffected in the current study, the mechanism behind this observed growth deficit is yet to be determined.

Gill damage as a result of turbidity exposure was expected to limit oxygen supply, theoretically resulting in a reduced aerobic scope, where oxygen uptake would struggle to provide for non-vital processes such as growth (Buraeu *et al.*, 2002). However, this theory was not sustained because AMS was not affected by turbidity so *P. auratus* should, from a metabolic sense, have the same growth potential across the 5 treatment groups. So why then, was the growth of turbidity treated fish reduced if no measure of metabolism was affected? The next logical test was to examine whether turbidity simply reduced the foraging ability (i.e. food gathering potential) of fish by direct visual impairment.
4.3.1 What are the potential factors that contribute to poor growth in turbid waters?

Changes in feeding behaviour as a result of turbidity exposure was explored in an attempt to explain the reduction in growth observed following turbidity exposure. Feeding behaviour of *P. auratus* was significantly impacted at relatively low, and perhaps environmentally relevant NTU levels (Chapter 3). These results serve to support the theory that turbidity may reduce fish growth through a reduction in feeding ability as opposed to impaired oxygen uptake. However, despite this evidence, it is thought that other factors aside from reductions in feeding ability may impact on growth. The methodology used during the growth trials meant that fish were confined to a tank and thus were presented with multiple opportunities to feed, unlike the fish in the feeding behaviour experiments. As such, feeding ability may not solely explain the weight loss observed, and thus other physiological mechanisms may be at play that have not been addressed in this study. For example, this study solely focused on the gas exchange function of fish gills, but fish gills are multifunctional (Evans *et al.*, 2005). Whilst providing an efficient surface area for gas exchange, they are also an important site for osmoregulation and nitrogenous waste excretion (Evans *et al.*, 2005).

Osmoregulation is a vital physiological process in fish that balances ion transfer between the water and the blood (Evans *et al.*, 2005). Previous studies have found evidence to suggest that fish experience osmoregulatory stress from exposure to suspended sediment (Au *et al.*, 2004, Wong *et al.*, 2013) and this is likely to also be the case for snapper. For example Na+, K+-ATPase activity and chloride cells of the gill lamellae in *E. coioides* were altered, indicating that fish were experiencing some form of osmoregulatory stress (Au *et al.*, 2004). Similarly, Wong *et al.* (2013) suggested that an increase in chloride cell density in orange spotted grouper exposed to suspended sediment may be evidence of osmotic stress. The gills also function to transfer ammonia from the blood to the water and if allowed to accumulate, ammonia can be extremely toxic to fish with many chronic effects, from biochemical and
structural changes to convulsions and death (Randall and Wright, 1987). Accordingly, ammonia production must be balanced with excretion but although there is no direct supporting evidence to date, damage to gill integrity through turbidity exposure may have potentially impacted on the effectiveness of ammonia removal. It is possible therefore that although no obvious changes were observed in oxygen uptake ability, alterations in osmoregulation and ammonia excretion may occur as a result of gill damage and cause sub-lethal effects, potentially affecting fish growth and productivity, specifically in snapper.

4.4 The Future of *P. auratus* in New Zealand

*P. auratus* is an ecologically and commercially important fish species for New Zealand (Francis, 1997, Parsons *et al*., 2014) with juveniles relying on shallow coastal waters, such as estuaries, harbours and coastal embayments, for nursery grounds (Hartill *et al*., 2003, Langley, 1993). These shallow coastal habitats are particularly susceptible to increases in suspended sediment (Morrison *et al*., 2009) and as such, juvenile *P. auratus* are likely to be vulnerable. The results from this study demonstrate some negative effects of turbidity on juvenile *P. auratus*, however, these results must be interpreted with caution when assessing the ecological relevance with regards to the concentration and duration of suspended sediment seen in the Hauraki Gulf as the marine environment is highly dynamic and variable through both space and time.

Future research needs to identify the mechanism that underlies the observed growth deficit in snapper but the take home message of this study is not altogether dire. If increased turbidity remained an episodic event driven by infrequent storm events, the fact that *P. auratus* are not oxygen limited as a result of gill structural change indicates that this species may be more resilient to environmental change than previously assumed. That gill structure is possibly
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maintained in excess of functional requirements opposes the theory of symmorphosis, but suggests that the anatomy of some fish species may have capacity to buffer oxygen uptake against the harmful effects of environmental change such as increases in suspended sediment concentrations (Randall and Brauner, 1991). Such resilience and environmental buffering capacity is likely to be of particular importance to species such as *P. auratus* that are subject to both commercial and recreational fishing pressure in a rapidly changing world. On the other hand, it is unknown whether or not the gills of juvenile *P. auratus* can recover from these turbidity-induced histopathological changes. If *P. auratus* are unable to recover, gill damage may accumulate, and with ongoing sediment pulses the gills of snapper may become progressively altered. There is currently no information regarding this aspect of fish gill morphology, so further studies may wish to address this gap in the knowledge and investigate thresholds of turbidity effects with respect to exposure duration and concentration.

Although *P. auratus* demonstrates a degree of resilience to turbidity with regards to oxygen uptake (Chapter 2), increased turbidity was shown to significantly affect the growth (Chapter 2) and feeding behaviour (Chapter 3) of snapper. Significant weight loss was observed at 40 NTU and while these NTU levels are observed in the Hauraki Gulf they may not necessarily be ecologically relevant. In order to assess any change in oxygen consumption, it was imperative to submit fish to long-term high-turbidity exposure to ensure the gill changes seen in Lowe *et al.*, (2015). However, although these levels are observed in the environment (Table 1), in reality they are not likely to persist for long time periods (i.e. 30 days or more), as high turbidity is more often seen in pulses (Morrison *et al.*, 2009). Therefore, although snapper may experience these levels on a sporadic basis, they are unlikely to be exposed consistently to high concentrations for prolonged durations. Therefore, to directly assess the condition of snapper in the Hauraki Gulf, it may be of interest for future research to better simulate environmental patterns of turbidity such as short-term, high-turbidity pulses that are
typically associated with storms (Morrison et al., 2009). Although the current results may not be directly ecologically relevant, they provide a base for future research and give an indication of threshold levels for *P. auratus*. Furthermore, with predictions of increases in turbidity with response to climate change (Willis et al., 2007), turbidity may become more sustained, meaning that the levels of NTU in the current study may be significant in future years.

Although changes in growth did not occur until 40 NTU, significant changes in feeding behaviour were seen as low as 20 NTU. Interestingly, this level is well within the range that is commonly seen in the environment and is in fact the average NTU for a number of monitoring sites in the Kaipara harbour (Table 1), an important nursery ground for juvenile snapper (Morrison et al., 2009). This result may therefore be of ecological consequence to populations in these areas and the feeding behaviour of *P. auratus* may be forced to change as a result of turbidity exposure. For example, Lowe et al. (2015) demonstrated that *P. auratus* switched from pelagic dominated prey such as calanoid copepods in higher clarity waters to benthic prey species in more turbid waters. This alteration in diet may reduce overall food availability for *P. auratus* as well as alter relative nutritional values (Lowe et al., 2015).

If turbidity in the Hauraki Gulf continues to deteriorate, these results could have significant ecological consequences for *P. auratus*. As juvenile *P. auratus* are known to occur in greatest numbers in shallow coastal and estuarine habitats (Parsons et al., 2014) where sedimentation effects are the greatest (Morrison et al., 2009), increased reliance on only a few critical estuarine habitats may render this species particularly susceptible to increases in turbidity. Reductions in feeding ability, coupled with impaired growth or weight loss may reduce the resilience of snapper populations, affect reproduction and alter the role that these species play in the environment, with potential flow-on effects for fisheries productivity (Morrison et al., 2009). It is also important to note that the impacts of suspended sediment described in the current study cannot be considered in isolation from other stressors such as
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hypoxia (Wu, 2002), overfishing (Jackson et al., 2001) and climate change (Willis et al., 2007), as well as loss of sea-grass and other important habitat, which are likely to interact in a synergistic way and pose a significant threat to these fished populations (Morrison et al., 2009). If these aquatic environments become progressively more turbid as a result of continuing land use changes and poor management, the relative importance of snapper and other fish species is likely to change with alterations in growth and population productivity.

4.5 CONCLUSION

Contrary to previous speculation, these results provide direct experimental evidence against the hypothesis that gill damage as a result of turbidity exposure impacts on the respiratory performance in juvenile *P. auratus*, providing evidence against the theory of symmorphosis. Our results also demonstrate that fish growth as a potential fitness measure is also impacted by rising turbidity levels but, whilst associated with gill structural change, it cannot be attributed to oxygen transfer deficits across the gills. Indeed, evidence was found to suggest that alterations in prey acquisition may contribute to the observed reduction in productivity, although this mode of impact is only likely to explain a portion of the weight loss. These results collectively suggest that turbidity can have negative effects on individual *P. auratus* and their fitness, which may translate into subsequent reductions in productivity at a population level. Acceptable threshold levels of turbidity for juvenile *P. auratus* may be as low as 20 to 30 NTU, as reductions in feeding ability and growth were seen at these levels. However, future research may wish to examine duration thresholds of turbidity to provide further insight for environmental relevance. Given the significance of turbidity as a stressor in the New Zealand marine environment, and the importance of snapper as a commercial and recreational fish species, this thesis provides valuable insight into some of the indirect physiological and behavioural impacts of turbidity. Such findings highlight the need for management to
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encompass both marine environments and terrestrial catchments for effective mitigation of turbidity and protection of valuable fish species. Additionally, this study provides scientific evidence to better inform decision makers for the management and monitoring of turbidity in New Zealand.


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