

**A methodology to develop aquaculture-based
release strategies with an application to *Metapenaeus
dalli* in the Swan-Canning Estuary: the Survival-
Maximisation-at-Release Tool
(SMART)**

Submitted by

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Declaration

I declare that the information contained in this Thesis is the result of my own research unless otherwise cited, and has as its main content work which has not previously been submitted for a degree at any university.

Kyle Philip Hodson

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Abstract:

Aquaculture-based enhancement, a method of releasing cultured organisms into the wild to boost fishery productivity, is becoming increasingly popular as a management option for restoring depleted fish stocks and increasing fishery yields. In the first section of this Thesis, I review and synthesise how the effectiveness of aquaculture-based enhancements can be optimised to maximise survival of released individuals. Two specific areas were identified that have a major influence on the short-term survival of released animals (i) “training” or acclimation in the hatchery to conditions in natural systems, *e.g.* habitats, water flows, natural (live) food and the smell and sight of predators through predator avoidance training and (ii) the development of a sound release strategy, involving the selection of site, time and size at release. The objective of the second section of this Thesis was to create a tool to inform the development of an optimal release strategy, by evaluating site selection and time of release for the release of post-larval Western School Prawns *Metapenaeus dalli* in the Swan-Canning Estuary. This was achieved by developing the Survival-Maximisation-At-Release-Tool (SMART), a quantitative tool that collated factors and variables considered to influence the survival of released *M. dalli* at potential sites around the estuary to determine a SMART score (0-100) for each potential release site and time (Month, Year, Day/Night). Statistical analyses on the resultant SMART scores determined that region of release was the most influential factor for the survival of released *M. dalli*, followed by year and then month. Due to the wide range of values for the salinity variable and sediment composition and predation factors among sites and time, these had the most influence on overall SMART score. Across the 16 nearshore sites sampled in five consecutive months in each of three years, the optimal site of release was at Deep Water Point in the Lower Canning Estuary during the night in January 2014. Recommendations for future improvements to the SMART methodology for releases of *M. dalli* were identified and mechanisms for adapting this tool for its application to other species and aquatic ecosystems

are discussed. The SMART provides output that can be readily conveyed to diverse audiences (*i.e.* fishers, researchers, managers and the community) to enhance discussions on optimal release strategies.

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Chapter 1: General introduction

Global landings from wild capture fisheries have plateaued since the late 1980s (Pauly *et al.*, 2002), with many stocks suffering from overexploitation and the adverse effects of habitat degradation (Lorenzen *et al.*, 2010). This, coupled with an expected increase in demand for fish protein as a result of increase in global population (FAO, 2012), highlights the need for immediate management intervention in order to manage existing fisheries sustainably so that fisheries yield can be stabilized, or more preferably, increased.

One method currently used to address this need to increase fisheries yield is the control of fishing effort through input controls such as spatial and temporal closures and gear restrictions, and output controls such as size limits and catch quotas. However, such interventions have the potential to place significant social and economic hardship on businesses and communities that previously had less restricted access to fish stocks (Mascia *et al.*, 2010; Bennett and Dearden, 2014). Another potential intervention that is becoming increasingly popular is the use of aquaculture-based enhancement, a method that involves the release of cultured juveniles into an environment to enhance, conserve or restore fisheries (Bell *et al.*, 2006; Lorenzen, 2008; Lorenzen *et al.*, 2010 Taylor *et al.*, 2016). This method can be separated into three major categories: (i) stock enhancement, the release of hatchery seed to improve self-sustaining populations; (ii) restocking, the release of hatchery seed to rebuild severely depleted fish stocks; and (iii) sea ranching, the release of hatchery seed in put and take operations (Bell *et al.*, 2008; Lorenzen *et al.*, 2013).

Success in aquaculture-based enhancement programs, generally referred to here as release programs, has the potential to yield significant social, economic and ecological benefits for fish stocks and their users. They could increase productivity beyond the level achievable by harvest management alone, create economic opportunities for fishery-livelihoods and provide incentives for active

management of fisheries resources (Pinkerton, 1994; Lorenzen and Garaway, 1998; Lorenzen, 2005). However, despite these potential benefits, along with significant investment in research and recent advances in the science of release programs, successes in establishing viable populations have been variable, often due to high mortality of released individuals shortly after release (Bell *et al.*, 2005; Lorenzen, 2005; Armstrong and Seddon, 2008; Vиллемey *et al.*, 2013). In order to reduce this post-release mortality, a more informed approach to the development and implementation of release programs is required.

Multiple examples of release programs have linked the reduction of post-release mortality to significant improvements in success (*e.g.* Leber 2002; Loneragan *et al.*, 2006; Taylor and Suthers, 2008; Zohar *et al.*, 2008). For example, for the release of Chesapeake Bay Blue Crab *Callinectes sapidus* in Northern America, it was determined that the survival of released crabs, their growth rate to sexual maturity and likelihood of successful integration into the spawning stock was a direct function of the well-researched and well-developed release program (Zohar *et al.*, 2008). Thus, designing a sound release strategy including the selection of release size, site and time is vital to reducing the post-release mortality of release organisms and thus maximising the success of the release program (Blankenship and Leber, 1995; Lorenzen *et al.*, 2010).

In Western Australia, a number of recent release programs have been funded using revenue generated from recreational fishing via the Recreational Fishing Initiatives Fund, a State Government grants program administered by Recfishwest. These release programs include, a three-year study to develop the aquaculture production and release of the Western School Prawn *Metapenaeus dalli* in the Swan-Canning Estuary and similar projects to release Mulloway *Argyrosomus japonicus* and Pink Snapper *Chrysophrys auratus* into nearshore coastal waters off the Perth metropolitan area and Blue Swimmer Crabs *Portunus amatus* in the Peel-Harvey Estuary. Therefore, successful release programs are crucial in order to provide good outcomes for the money spent by recreational

fishers. This success requires careful consideration of the release strategy, including the selection of release site, time and size-at-release, a step that is required under recent revision of the Policy on Restocking and Stock Enhancement in Western Australia (Department of Fisheries, 2013).

The first section of this Thesis (Chapter 2) builds upon previous work on the development of responsible approaches to release programs (including release strategies) particularly Blankenship and Leber's (1995) 'Responsible Approach to Marine Stock Enhancement' and a subsequent revision by Lorenzen *et al.* (2010). These studies identify the key components of release programs that affect short-term, post-release survival of hatchery-reared animals, including techniques used within the hatchery, the selection of site, size and time-of-release and methods used to assess the success of releases.

The second section of this Thesis (Chapter 3) focuses on creating an objective, quantitative tool to assist in selection of suitable site and time for the release of hatchery-reared juvenile *Metapenaeus dalli* in the Swan-Canning Estuary. This tool incorporates those factors identified in the Chapter 2 as being influential in the short-term survival of *M. dalli* and thus should be considered in the selection of a release site and time, combining them in such a way to develop an overall evaluation for each potential release site within the estuary. Data for this evaluation was collated from a variety of sources, in particular, from a research program focusing on the restocking of *M. dalli* and evaluating recruitment limitation, environment and release strategies undertaken by Murdoch University staff and students.

To complete the above overall objectives, my Thesis has the following aims:

1. Conduct a literature review to identify the main components of release programs and the factors influencing the survival of released individuals, in particular those that affect the choice of release site and time (Chapter 2).
2. Determine those abiotic and biotic factors that will likely have an impact on the short-term survival of released post-larval *M. dalli* (Chapter 3).

3. Determine the spatial and temporal variation in those factors identified as potentially being influential for the survival of post-larval *M. dalli* within the Swan-Canning Estuary (Chapter 3).
4. Develop a methodology to collate and synthesise quantitative data on the factors affecting post-release survival at each site to provide an objective, quantitative basis for selecting release sites for post-larval *M. dalli* in the Swan-Canning Estuary, *i.e.* the Survival-Maximization-at-Release-Tool (SMART; Chapter 3).

Chapter 2: Maximising the success of aquaculture-based enhancements: Considerations to improve survival in the hatchery and the wild

2.0: Abstract

With landings from world capture fisheries reaching a plateau and the looming pressure of increasing populations on recreational fish stocks, release programs of cultured fish, termed stock enhancement and restocking, are being seen increasingly as a management intervention of choice. This literature review builds on previous syntheses of responsible stock enhancement, firstly Blankenship and Leber's (1995) 'A Responsible Guide to Stock Enhancement' and secondly the subsequent revision and update of this paper by Lorenzen *et al.* (2010). It addresses key components for consideration when designing a release strategy, using select case studies, focusing mainly on marine releases, but also drawing on lessons learned from freshwater and terrestrial programs, to discuss how release programs should be managed in order to optimise their success. All areas of a release program have been considered, beginning with culturing techniques for use in the hatchery to prepare juveniles for release, then looking at release strategies; choosing the optimal site, time (*i.e.* diurnal/seasonally) and size for release, as well as the importance of care in transportation to release site and appropriate acclimation immediately prior to release, and finally identifying the monitoring and evaluation techniques available to evaluate the success of a release program. The review also identifies the social and economic benefits and costs posed to a community by release programs, how to ensure that adverse effects of these components are minimised and benefits maximised. Information from the review is then summarised into 11 key components that I regard as necessary for consideration when undertaking a release program to ensure that success of the program is optimised and program goals are being met.

2.1: Introduction

Global fishery resources have plateaued since the late 1980's (Pauly *et al.*, 2002) most likely as a result of habitat degradation and over exploitation (Jackson *et al.*, 2001; Brown and Day, 2002). In 2012, the Food and Agriculture Organisation [FAO] (2012) identified that ~30% of global fish stocks were overexploited, *i.e.* below the size that produces maximum biological and ecological potential, with a further 28% near their maximum sustainable yield or fully exploited. Only 12% of stocks were found to be underfished and have the carrying capacity to survive without immediate management intervention. Pauly *et al.* (2002) and, more recently, Worm and Branch (2012) suggested that if global fish stocks were to recover, a significant reduction in fishing effort was needed and that ~20% of marine environments should be designated as sanctuary areas. These actions, however, would cause serious economic and social hardship on those businesses and communities relying on fisheries, thus highlighting the need for additional management interventions (Mascia *et al.* 2010; Bennett and Dearden, 2014).

In addition to regulating fishing effort to ensure sustainability, release programs present a complimentary management strategy to rebuild depleted fish stocks or augment stocks (Hilborn, 1998; Travis *et al.*, 1998; Leber, 2002; Bell *et al.*, 2005, 2008). Release programs can be subdivided into a number of categories namely (i) restocking, *i.e.* the release of cultured juveniles into wild populations to restore depleted spawning biomass to a level where it can once again provide regular, substantial yields, (ii) stock enhancement, *i.e.* the release of cultured juveniles into wild population(s) to augment the natural supply of juveniles and optimise harvests by overcoming recruitment limitation, and (iii) sea ranching, *i.e.* the release of cultured juveniles into unenclosed marine and estuarine environments for harvest at a larger size in 'put, grow and take' operations (Bell *et al.* 2005, 2008; Leber, 2013; Vилlemey *et al.* 2013).

Despite its obvious attraction as a mechanism to increase fisheries production and rebuild fish stocks, the performance of release programs has been

mixed and, more often than not, disappointing (Bell *et al.*, 2005; Lorenzen, 2005). Many enhancements have failed to significantly increase a fishery's yield, provide economic benefits and/or have had detrimental effects on the natural population of the targeted species (Hilborn, 1998; Levin *et al.*, 2001; Arnason, 2001). Hence, the need for a more informed and responsible approach to the development of release programs has been widely recognised in freshwater and marine systems (*e.g.* Cowx, 1994; Lorenzen and Garaway, 1998). Blankenship and Leber's (1995) 'A Responsible Approach to Marine Enhancement', outlined a recommended approach for developing, evaluating and managing marine stock enhancement in a responsible way, which was later re-evaluated by Lorenzen *et al.* (2010) (Table 2.1). The former paper identified a set of principles considered essential to control and optimise enhancement, with Lorenzen *et al.* (2010) refining and updating what has become the 'Responsible Approach' to stock enhancement in light of developments in fisheries science and management in the 15 years since the first paper. This approach recommends comprehensive pre- and post-release studies into those factors that are likely to influence survival and thus must be considered when designing a release strategy (Villemey *et al.*, 2013).

As discussed by Lorenzen (2005), the likelihood of success is difficult to predict for many release programs. This review aims to identify components of a release program that are considered necessary to address in order to optimise the success of a release program. A broad spectrum of case studies from marine, freshwater and terrestrial release programs, on both short-lived and long-lived species, considered good examples of release programs will be used to support these criteria, including features such as the presence of long term monitoring and multi-disciplinary studies. It will also identify how adverse effects may be avoided in release programs and summarise the criteria and methods used to evaluate the success of releases after stocking or post-release. In doing so, four key criteria will be outlined for the development of an optimal release program: Improving hatchery survival (including transport to the release site, Section 2); improving

survival in the wild (Section 3); maximising social and economic benefits (Section 4); measuring success (Section 5) and conclusions (Section 6), including 11 recommendations for optimising success.

Table 2.1: Comparison of the key principles for responsible stock enhancement identified by Blankenship and Leber (1995) (left) and Lorenzen *et al.* (2010) (right).

Blankenship & Leber (1995)	Lorenzen <i>et al.</i> (2010).
Prioritise and select target species for enhancement	Understand the role of enhancement within the fishery system
Develop a species management plan	Engage stakeholders and develop a rigorous and accountable decision making process
Define quantitative measures of success	Quantitatively assess contributions of enhancement to fisheries management goals
Use generic resource management	Prioritise and select target species for enhancement
Use disease and health management	Assess economic and social benefits and costs of enhancement
Form enhancement objectives and tactics	Define enhancement system designs suitable for the fishery and management objectives
Identify released hatchery fish and assess stocking affects	Develop appropriate aquaculture systems and rearing practices
Use an empirical process to define optimum release strategies	Use genetic resource management to maximise effectiveness of enhancement and avoid deleterious effects on wild populations
Identify economic and policy objectives	Use disease and health management
Use adaptive management	Ensure that released hatchery fish can be identified
	Use an empirical process for defining optimal release strategies
	Devise effective governance arrangements
	Define a fisheries management plan with clear goals, measures of success and decision rules
	Assess and manage ecological impacts
	Use adaptive management

2.2: Improving survival in the hatchery

2.2.1: Aquaculture methods

Animals reared in aquaculture facilities are housed in a predator-free environment and provided with a surplus of food. While such conditions result in higher survival rates relative to the wild, particularly in the vulnerable early life stages, they do not reflect natural systems and thus the transition from living in a hatchery to surviving in the wild can be a time of high mortality for released

individuals (Fleming and Einum, 1997; Hard *et al.*, 2000; Araki *et al.*, 2008; Burns *et al.*, 2009; Mayer *et al.*, 2011). Hatchery-reared fish often perform poorly in the natural environment (Jonsson *et al.*, 2003; Saloniemi *et al.*, 2004; Kallio-Nyberg *et al.*, 2006; Serrano *et al.*, 2009) and exhibit high mortality rates, especially in the first few days after release (Olla *et al.*, 1998; Brown and Day, 2002). Numerous studies have demonstrated that this higher mortality is due to behavioural deficits in the hatchery reared stock that can be improved by 'conditioning' in the aquaculture facility (Cresswell and Williams, 1983; Svåsand *et al.*, 1998; Tsukamoto *et al.*, 1999; Svåsand, 2004). Conditioning, to increase the potential survival of animals post-release and acclimating them to more natural conditions, has included strategies such as enriching habitats, adjusting food to include prey likely to be encountered in the natural environment and introducing cultured individuals to predators. Each of these facets of hatchery conditioning to natural environments is discussed below.

Habitat enrichment

In an aquacultural context, habitat enrichment involves increasing the environmental complexity of the hatchery to reduce maladaptive and aberrant traits found in animals reared in stimuli-deprived environments. This approach has been used, on cultured individuals, to develop behaviours that are similar to those exhibited by individuals in the natural population (Näslund and Johnsson, 2016). Habitat enrichment has been shown by Näslund *et al.* (2012) to reduce the short-term mortality rates of cultured juveniles post-release, easing adaptation to the natural environment and promoting the development of skills such as foraging and predator-avoidance. These authors investigated the effect of enrichment on the brain-development of hatchery reared Atlantic Salmon *Salmo salar*, while numerous studies have been conducted on other species (*e.g.* Kempermann *et al.*, 2002; Kerridge, 2005; Kihslinger and Nevitt, 2006).

Juvenile fish respond quickly to environmental conditions and can exhibit phenotypic differences. For example, Pakkasmaa and Piironen, (2001) found that

the body shape of juvenile Atlantic Salmon and Brown Trout *Salmo trutta* differed when the fish were reared in fast or slow moving water. Therefore, it could be expected that hatchery-reared individuals, without conditioning to water flow, may be phenotypically mismatched to natural environments characterised by high flows. Stringwell *et al.* (2014) tested this theory by culturing first generation juvenile Atlantic Salmon under standard hatchery conditions, *i.e.* without habitat enrichment, releasing 0+ fry into four rivers and retaining a subsample of fish in the hatchery as a control. After 20 days, fish recaptured from the wild had become more cryptic in their colouration and had undergone changes in their body shape, being more streamlined, more symmetrical and having developed longer heads and thicker caudal peduncles than fish still in the hatchery. These differences in phenotype became even more marked 55 days post-release (Stringwell *et al.*, 2014). Flowing water was also used in the acclimation of juvenile Qingbo *Spinibarbus sinensis* (Fu, 2015). It was found that those fish that were exposed to flowing water swam faster for prolonged periods and were three times more likely to avoid predation in comparison to fish raised under standard hatchery conditions (still water; Fig. 2.3).

Differences in body shape (*i.e.* length or dorsal spine; Fig. 2.1), colouration and predator avoidance were also detected between cultured and wild Blue Crabs *Callinectes sapidus*. However, exposing the crabs to sediment in the natural environment for just two days resulted in a reduction in intensity of blue colour and an increase in burying activity so that these characteristics no longer differed significantly from those of the wild crabs (Davis *et al.* 2005; Young *et al.*, 2008). Interestingly, crabs maintained in the laboratory for 30 days, whether cultured or wild, had shorter spines than natural crabs that had never been held in culture. However, exposure of hatchery crabs to a predatory fish resulted in increased development of the spines so that they did not differ in length from crabs in the natural environment (Young *et al.*, 2008). These results and measurements of the survival of conditioned, unconditioned and wild crabs led Young *et al.* (2008) to

conclude that, while morphological and behavioural differences were detected in wild and cultured crabs, conditioning rapidly mitigated these differences to the extent that any remaining differences did not translate into decreased survival post-release.

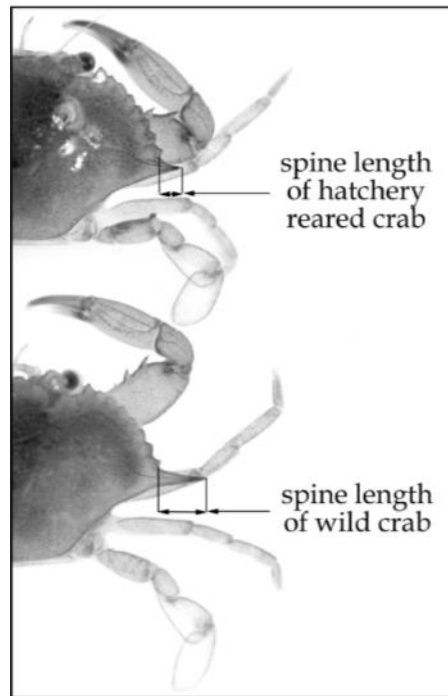


Figure 2.1: Differences in morphology between hatchery-reared (top) and wild-caught (bottom) Blue Crabs, *Callinectes sapidus*, in Chesapeake Bay. Taken from Young *et al.* (2008).

Food and feeding

Fish living in the hatchery environment are regularly provided with an abundance of easy to catch prey items, typically pelletised food and are conditioned to feed at regular times of day. In contrast, in natural environments, food is not evenly distributed nor easily obtained. As a result, when cultured juveniles are released into the wild, they often forage inefficiently, which can lead to high rates of mortality. Laboratory studies demonstrated that Atlantic Cod *Gadus morhua* cultured in ponds on a diet of pellets, fed differently to wild individuals with cultured Cod catching prey fish after swimming pursuits, while wild Cod caught prey in lunges (Steingrund and Fernø, 1997). Although this study indicated that cultured Cod were able to catch prey fish, pursuit swimming has far

higher energy costs than lunge feeding and also exposes the juvenile Cod to predators for longer durations during the pursuit.

After release into the wild, captive reared Atlantic Salmon exhibited lower foraging rates on natural prey and thus had lower survival and growth rates than wild-born conspecifics (Rodewald *et al.* 2011). However, Roberts *et al.* (2014) found that exposing cultured fish to habitat enrichment, through the introduction of tree branches and cargo netting to the hatchery environment, resulted in these fish feeding more often and starting to feed sooner than fish in standard hatchery environments (Fig. 2.2). They concluded that this form of conditioning may reduce differences in feeding strategies between cultured and wild individuals and, in turn increase the survival of cultured fish. Similarly, Rodewald *et al.* (2011) conditioned juvenile Atlantic Salmon in an enriched environment, featuring submerged overhead shelter, varying water current, depth and direction and consequent alterations in food dispersion, found that survival increased compared with unconditioned fish.

A lack of experience in feeding on natural prey has also been cited as a contributing factor to the reduced survival of released fish. For example, only 42% of cultured juvenile Turbot *Schophthalmus maximus* consumed live shrimp on first encounter, suggesting that these fish did not consider live shrimp to be a prey item (Donadelli *et al.*, 2015). The results from studies of stomach fullness of Atlantic Salmon and Fry reared in environmentally enriched habitats and under regular tank conditions showed a marked decrease in the proportion of empty stomachs in those fish reared in the enriched environment compared to those reared in control tanks (Fig. 2.2, Roberts *et al.*, 2014). One method of reducing difference in feeding success between cultured and wild individuals is to expose cultured fish to live prey items. When juvenile Atlantic Salmon were cultured with live bloodworms, they were more likely to feed on other live prey items *i.e.* Brine Shrimp, even though they had not previously encountered them (Brown *et al.*, 2003a). Likewise, Norris (2002) found that after 30 days of feeding on a live food diet, Whiting

Sillaginidae spp. were significantly faster at locating prey in the wild than pellet fed fish. Other studies have also demonstrated that cultured fish could be trained to accept live prey from the surface or from the benthos, which dramatically increased foraging success (Brown *et al.*, 2003b). Moreover, if naïve fish were able to view trained fish feeding on live prey for six days they learnt this skill and exhibited a greater foraging efficiency.

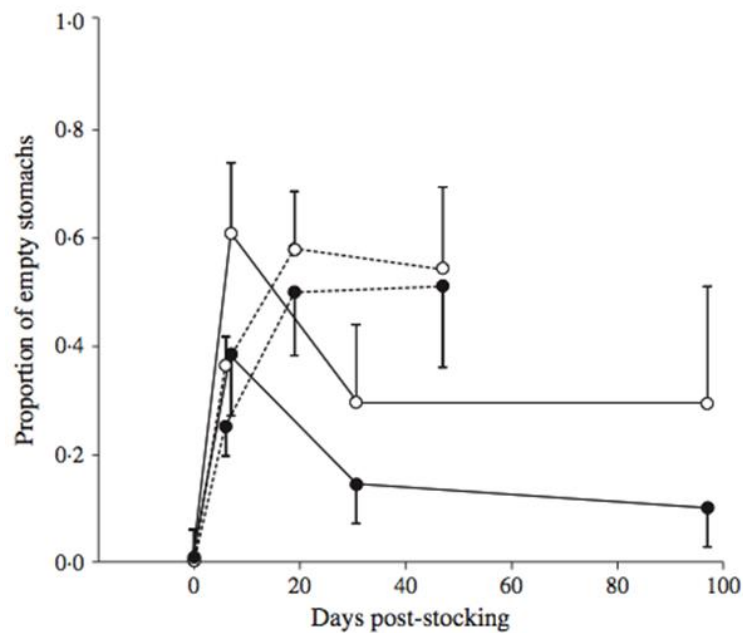


Figure 2.2: Temporal variation in the mean and 95% confidence limits for the proportion of empty stomachs of Atlantic Salmon, *Salmo salar* (dashed line) and fry (solid line) reared under control (○) and environmentally enriched (●) conditions prior to release (Roberts *et al.*, 2014).

Predator Avoidance

Hatchery rearing has also been suggested to limit the behavioural, morphological and physiological mechanisms of cultured individuals for recognising and avoiding predators (McLean *et al.*, 1996; Sutherland, 1998; Einum and Fleming, 2001; Knight, 2001; Mathews *et al.*, 2005). A number of studies have found that hatchery-reared individuals suffer a higher predation rate than their wild conspecifics (*e.g.* Stunz and Minello, 2001; Brown and Laland, 2001; Brown and Day, 2002; Young, *et al.*, 2008). Whilst wild juveniles have innate predator avoidance skills, triggered through successive encounters with predatory species (Kelley and Magurran, 2003), cultured juveniles are often released with no prior

experience or exposure to predatory species (Olla and Davis, 1989; Olla *et al.*, 1998; Wallace, 2000). In salmonids, such as Atlantic Salmon and Brown Trout, hatchery-reared individuals display riskier behaviour (Johnsson, *et al.*, 1996) and delayed physiological and behavioural responses to predators than wild fish (Johnsson, *et al.*, 2001; Hawkins, *et al.*, 2004). However, cultured juveniles exposed to predators within the hatchery developed greater predator avoidance abilities (*e.g.* Dill, 1974; Magurran, 1990; Berejikian, 1995; Brown and Smith, 1998; Mirza and Chivers, 2000; Vilhunen *et al.*, 2005).

The effect of predatory avoidance training and conditioning have been tested on juvenile Atlantic Cod in Norway (Svåsand, 2004). Juveniles were trained by placing them with larger Cod and conditioned by being held in the wild, in a seine net, for two days prior to release (Otterå *et al.*, 1999, Svåsand, 2004). Both training and conditioning were shown to significantly increase survival compared with the control group (untrained and unconditioned). Fu (2015) investigated the impact of predator acclimation on juvenile *S. sinensis*. Fish exposed to predators reacted more quickly to predator stimuli than control fish (Fig. 2.3). With treatment resulting in an approximately three times greater success rate in avoiding predators (success rate of avoidance \approx 60%) and hence lower mortality from predation than the control groups of fish (success rate of avoidance \approx 18%, Fig. 2.3).

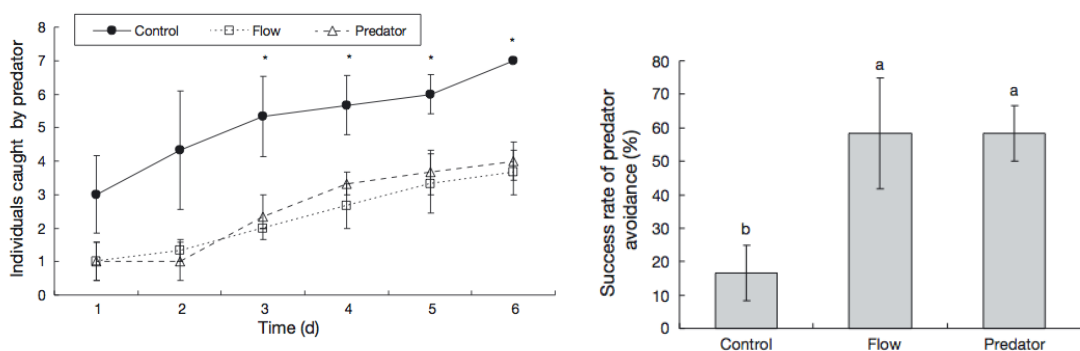


Figure 2.3: (Left) The number of Qingbos, *Spinibarbus sinensis*, consumed by predators after different periods of predator exposure (mean \pm SE). * denote significant differences among groups ($p < 0.05$) and (right) the mean success rate of predator avoidance (± 1 SE) by Qingbos after 20 d of different experimental acclimation conditions. Taken from Fu (2015).

Predation by piscivorous birds may also be an important cause of mortality in post-release juveniles (Brown and Day, 2002). In an attempt to reduce this source of mortality, Hutchinson *et al.* (2012) used dead cormorants to simulate a chasing behaviour in the tank environment, encouraging Murray Cod *Maccullochella peelii* to seek shelter in patches of artificial weed placed around the tank by harassing fish in open areas. The use of cover and tendency to move away from the simulated bird attacks by the fish increased significantly after their first exposure. This technique is likely able to be applied also to invertebrates, as studies into their behavioural escape response have been analysed (Herberholz and Marquart, 2012).

Whilst many of the above studies used live predators to help cultured fish develop evasive behaviour, scents have also been used successfully to pre-condition juveniles and enhance predator avoidance skills (*e.g.* Fuiman and Magurran, 1994; Brown and Smith, 1998; Berejikian *et al.*, 1999; Mirza and Chivers, 2000). For example, Vilhunen (2006) conditioned hatchery-reared Arctic Charr *Salvelinus alpinus* with the odour from Pikeperch *Sander lucioperca*, which had been fed with Arctic Charr. Juveniles exposed to the Pikeperch odour on only a single occasion made significantly less direct approaches toward the odour source than those not previously exposed to the predator's odour and their post-release survival was similar to that of fish given direct experience with a predator.

While this study and several others (*i.e.* Mathis and Smith, 1993; Chivers and Smith, 1998; Gazdewich and Chivers, 2002), have shown that a single exposure to predator stimulus is enough to enhance predator avoidance, it is noteworthy that other experiments have demonstrated that avoidance skills learnt in the hatchery can decrease over time in captivity (McLean *et al.*, 1996; Brown and Smith, 1998; Berejikian *et al.*, 1999). Therefore, repeating predator avoidance, pre-conditioning or training overtime may equip hatchery reared juveniles with stronger and longer-enduring responsiveness and thus increase survival in the wild (Vilhunen, 2006).

2.2.2: Transport to release site

Transport and handling of the cultured individuals from the hatchery to the wild can be stressful and result in fish and invertebrates dying or becoming far more vulnerable to predation upon release (Simpson *et al.*, 2002; Portz *et al.*, 2006; Purcell *et al.*, 2006). Although some animals are particularly hardy, it is important to determine the optimal stocking density and transport duration that can be comfortably sustained by a species prior to release without producing adverse effects (Purcell, 2004). Transport in static conditions for long durations can result in the buildup of toxic waste products in the water. Thus, water temperature is often reduced to slow down metabolism and lower oxygen consumption and excretion by the animals in transport (Amend *et al.*, 1982). Alternatively, fish have been starved for 24 h before transportation to reduce oxygen demand and ammonia build up during transport (Cowx, 1994). For some gastropods, such as *Trochus spp.* and *Haliotis spp.*, damp cloths are more effective transport media than water (Dobson, 2001; Heasman *et al.*, 2003).

Upon arrival at a release site, Simpson *et al.* (2002) recommend that fish be acclimatised prior to release, allowing adjustment to changes in water quality to occur, which reduces stress and thus lowers short-term mortality from predation. Such acclimatisation has also been suggested for the release of sea cucumbers *Holothuria scabra* in New Caledonia and juvenile European Lobster *Homarus gammarus* in Norway (Purcell *et al.* 2006; van der Meeren 1991, 1993). When juvenile lobsters were transported in waters of 2-5 °C and released without acclimatisation in a warmer ambient sea temperature (~12 °C), they remained immobile for several minutes. This resulted in high immediate post-release mortality with > 10% of the juveniles being consumed within the first few hours. However, lobsters acclimatised to the ambient seawater temperature prior to release moved with ease and thus short-term mortality decreased (van der Meeren 1991, 1993).

2.2.3: Disease Transfer

Disease transfer from hatchery-reared fish to wild stocks is a major risk associated with release programs (Blankenship and Leber, 1995; Lorenzen *et al.*, 2010). The most dramatic examples of disease impacts associated with release programs of hatchery-reared fish have been caused by the introduction of alien pathogens (*e.g.* Johnsen and Jensen, 1991; Wagner, 2002), likely resulting from movements of cultured stocks, because the ranges of hosts and parasites are not necessarily identical (Johnsen and Jensen, 1991). Cultured fish may also increase the reservoir of susceptible hosts substantially where enhancements are carried out on a large scale. Thus, procedures for managing the health of hatchery fish are important and should include, as a minimum, health screening prior to release (Lorenzen *et al.*, 2010). For example, in Western Australia, cultured animals must be tested for nominated diseases by veterinary officers from governmental laboratories prior to their release into the wild (Department of Fisheries, 2004).

2.3: Improving survival in the wild

In many instances, the majority of mortality in post-release individuals occurs within the first few days after release, rather than spread over time (Olla *et al.* 1994; Brown and Laland 2001; Sparrevohn and Støtrup 2007). Therefore, careful consideration is required when developing a release strategy so that the effect of factors, such as the release site (Leber *et al.*, 1998; Gardner and Van Putten, 2008; Hines *et al.*, 2008), release time, *e.g.* season (Leber *et al.*, 1997; 1998; Hines *et al.*, 2008) and size-at-release (Leber *et al.*, 2005), are considered and the mortality rates of released juveniles can be reduced. Important in the long term success of a release program is the reduction in possible adverse effects through the mixing and dilution of genetic diversity within the population, hence appropriate precautions must be taken when undertaking broodstock collection.

2.3.1: Release site

Optimal release sites have few predators and competitors, ample shelter and an abundance of food (Hines *et al.*, 2008). When identifying an appropriate release site, knowledge of the biology and ecology of the species to be released is crucial, as well as ensuring that the proposed area is suitable. Below are examples of the key components that should be taken into consideration when selecting a site for the release of cultured juveniles.

Ecosystem

In instances where multiple ecosystems are available for the release of a species, the choice of ecosystem can have a significant effect on the survival of released juveniles. Lake Tyers in Victoria, Australia is an estuarine system at the southern limit of the distribution of the Eastern King Prawn *Penaeus (Melicertus) plebejus*. The recruitment of this species into Lake Tyers from its spawning grounds north of the Queensland border is very sporadic as it depends on the strength of the East Australian Current and whether the entrance to Lake Tyers is open to the sea (Taylor and Ko, 2011). However, in some years good catches of prawns are taken from Lake Tyers, which indicates that it has potential as a site for a successful release program, particularly in years when natural recruitment is low or does not occur. In recent years, the increased frequency and persistence of entrance closure caused limited recruitment into the system and it was decided that stocking directly into Lake Tyers, rather than into the ocean with the wild larval population was the most effective approach to maximising survival of released prawns (Taylor and Ko, 2011). By taking this approach, scientists ensured that the released prawns could reach nursery habitat, optimising their chance of survival.

Environmental conditions

Whilst a large number of fish species are capable of tolerating a wide range of physico-chemical conditions, optimal conditions can remove potential adverse effects and promote growth and survival of cultured fish (Bœuf and Payan, 2001).

These conditions include water temperature and salinity, the amount of dissolved oxygen present and the amount of phytoplankton present in the water column. Adverse environmental conditions can therefore result in a large number of detrimental effects on the survival of released individuals. For example, the growth rates of Atlantic Cod *Gadus morhua* were measured under different salinity conditions to determine the optimal level for growth and survival (Lambert *et al.*, 1994). Results showed that the Cod grew up to 63% faster at a salinity of 14 ppt than 28 ppt and thus it was suggested that they should be released into estuaries where/when salinities were brackish to optimise their growth and survival.

Prey availability

The availability of prey items is an important factor for the survival of post-release individuals and understanding the abundance of food at the release sites can help determine the appropriate release densities to ensure that food does not become a limiting factor. Dietary studies to determine preferred prey items of a species at different life stages and/or body sizes and consumption rates, such as that undertaken by Taylor and Suthers (2008) on Mulloway *Argyrosomus japonicas*, can assist in selecting a release site with sufficient food sources. These authors created a predation impact model to evaluate potential release densities and the carrying capacity of the environment for Mulloway in the Georges River Recreational Fishing Haven, Sydney. This was done to model whether prey densities were abundant enough to support a release of 17,500 juvenile Mulloway and the predatory impact that the released fish were likely to have. In the first 3.5 years post stocking the predatory impact of a single Mulloway on prey was estimated to require one litre of mysids, 80,000 forage fish, 45,000 prawns, 3,000 miscellaneous invertebrates and 5,000 cephalopods.

Habitat

Key habitats must also satisfy the refuge requirements, especially for juveniles, in order for individuals to escape predation, forage efficiently and offer preferable physical conditions (Blaber, 1997; Taylor, 2006). In a study to

determine the habitat preference for Mulloway in south-east Australia, fish were released into an estuary containing numerous habitat types, such as discrete holes and basins up to 20 m deep. Nine hatchery-reared and 12 wild-caught Mulloway were acoustically tracked over 72 h in a 15 km section of the river. The results demonstrated that Mulloway preferred the deep hole habitat as small fish (300-500 mm TL), remaining in the holes during both day and night, while large fish (500-800 mm TL) ventured outside the deep holes at night. Smaller fish released in shallow waters travelled up to 10 km over the 72 h, whilst those released directly over deep holes moved far less. As a result, it was determined that juvenile Mulloway should be released directly into deep holes to minimise movements and thus reduce exposure to predation.

The densities and survival of juvenile Tiger Prawns *P. esculentus* and *P. semisulatus* are higher and growth rates faster on vegetated habitats (seagrass and macroalgae) than bare substratum (Kenyon *et al.*, 1995; Liu and Loneragan, 1997; Loneragan *et al.*, 1998; 2006; 2013). Simulations of the survival of released prawns in different nursery habitats showed that survival to the size at emigration from shallow waters (~15 mm carapace length and 12 weeks old) was nearly two times greater on high biomass seagrass (~100 gm⁻²) than on lower biomass seagrass beds (~10 gm⁻²) and bare sand (Loneragan *et al.*, 2006). These results clearly show that submerged aquatic vegetation is the preferred release habitat for hatchery-reared Tiger Prawns.

Predation

Predation is understood to be the single greatest hurdle in short-term post-release survival of cultured juveniles (Hines *et al.*, 2008; Støttrup *et al.*, 2008). For example, Buckmeir *et al.* (2005) estimated that after just 12 h, ~27.5% of released Largemouth Bass *Micropterus salmoides* were taken by predators, compared to mortality in predator-free enclosures of only ~3.5% after 84 h. Sites or habitat types that contain low abundances of identified and potential predators should be selected for release in order to reduce short-term mortality. A program to release

Sandfish *Holothuria scabra* [a sea cucumber] in the Solomon Islands found that differences in habitat had a significant effect on the survival of released individuals (Dance *et al.*, 2003). Sandfish released into mangrove/seagrass areas had a significantly higher survival than those released onto a coral-reef flat. Variations in fish faunas between these habitats were thought to be attributable, as four of the five fish families observed to have preyed upon Sandfish were recorded only on coral-reef flats. The significance of predators has been recognised in the release of Japanese Scallops *Patinopecten yessoensis* where predators are removed from the wild before seeding scallops (Bell *et al.*, 2005) as part of a very successful release program that has rebuilt this fishery. This operation is more a form of sea ranching than enhancement but demonstrates the significance of predation as a controlling factor.

Stocking density and competition

The term 'carrying capacity' refers to the inherent maximum population size of a species that an area can support given the amount of food, shelter and other necessities it can provide (Solomon, 1985). If wild juvenile stocks do not meet the carrying capacity of their habitat, the area may have capacity to support greater numbers of juveniles introduced through a release program. In contrast, if the population densities are high and close to or above the carrying capacity, released individuals are likely to suffer intense intra-specific competition with the wild population, leading to slower growth and lower survival (Aprahamian, *et al.*, 2003; Ochwada-Doyle *et al.*, 2012).

Ochwada-Doyle *et al.* (2012) examined the potential of competition for food and shelter between hatchery-reared and wild Eastern King Prawns and the resulting demographic density-dependence in Merimbula Lake on the southeast coast of Australia. Results from the experiments showed that when both food and shelter were limited, the survival rate of both cohorts was lowered equally. When food was limited, the survival rate of each cohort was unaffected by the addition of the other cohort, however, when wild Eastern King Prawn were added to a tank

with hatchery-reared prawns and shelter was limited, significantly higher mortality ensued in the cultured population. Likewise, when hatchery-reared prawns were added to a tank with wild prawns and shelter was limited, the hatchery-reared prawns suffered a higher-mortality. From these results, Ochwada-Doyle *et al.* (2012) concluded that under wild conditions, competition for shelter between the wild competition and released Eastern King Prawn may lead to the loss of hatchery-reared prawns, reducing the success of the release program.

Broadley (2014) investigated the effects of restocking Western School Prawn *Metapenaeus dalli*, in the Swan-Canning Estuary using the EnhanceFish model, a bioeconomic model that has separate components and parameters for the wild stock and the released. The results predicted that the introduction of cultured prawns would cause the biomass of wild prawns to decrease (Fig. 2.4) due to the density-dependent effects of increased competition between the released and wild populations for food and shelter, a common result of release programs (*e.g.* Molony *et al.*, 2003; Støttrup and Sparrevohn, 2007).

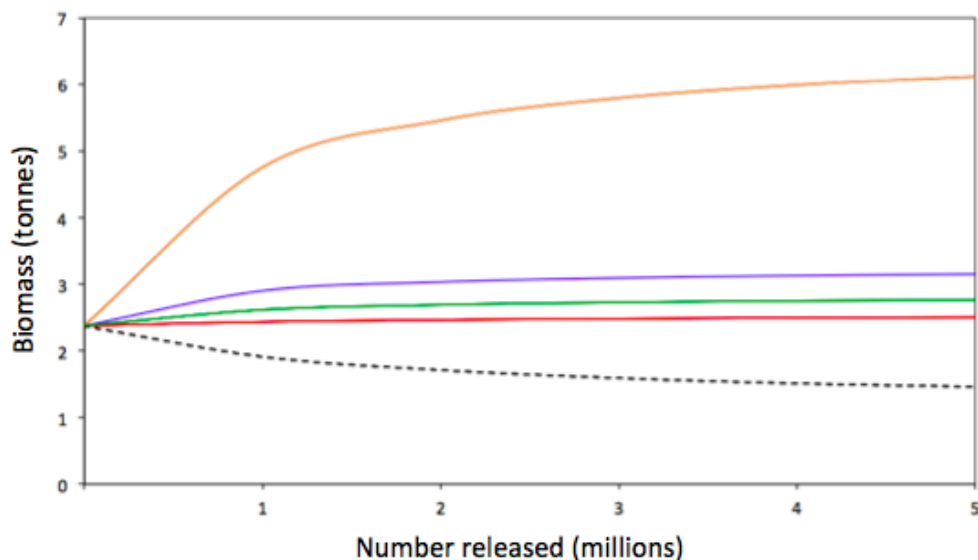


Figure 2.4: Change in biomass of *Metapenaeus dalli* population in the Swan-Canning Estuary with the number of prawns released and different sizes at release over a 5-year period. Wild population = black dashed line, 1 mm = red, 3 mm = green, 5 mm = blue, 10 mm = orange. Taken from Broadley (2014).

Intraspecific competition may also occur solely between individuals of the restocked population. For example, a study conducted into the optimal stocking density of Rainbow Trout *Ocorhynchus mykiss* determined that stocking at a density of 120kg/m³ resulted in a weight ~30% lower than that recorded when fish were stocked at a density of 25 kg/m³ (Person-Le Ruyet, *et al.*, 2008).

Released individuals may also experience inter-specific competition with other species sharing the same resources. For example, a study on the performance of hatchery-reared Brook Trout in six lakes across the Laurentian Shield, Canada observed that increase in weight and yield of Brook Trout were inversely correlated with the occurrence of White Sucker *Catostomus commersonii*. These results indicated that inter-specific competition between the released Brook Trout and White Sucker is likely to be a significant factor influencing success of the release program (Tremblay, 1991).

A release site should be selected that will provide the hatchery-reared species with environmental conditions and habitat that promotes growth and survival. Furthermore, it should contain a low abundance of predatory species and a high abundance of prey items. Sites should also be chosen that will have the maximum benefit to the aims of the release program (see ecosystem section) and finally, juveniles should be released at densities that do not exceed the carrying capacity of the selected release site(s).

2.3.2: Size-at-release

Size-at-release is considered an important factor in mitigating post-release mortality (*e.g.* Bilton *et al.*, 1982; Svåsand and Kristiansen, 1990; Leber, 1995; Leber *et al.*, 1997; Ye *et al.*, 2005). However, determining an optimal size for release can be challenging as, although larger individuals suffer lower mortality rates in the wild (Salminen *et al.*, 1994, 1995; Kallio-Nyberg *et al.*, 1999; Lorenzen, 2005), unless conditioned, longer culture times may result in these animals becoming adapted to the hatchery conditions and thus maladapted to the wild (*e.g.* Kellison *et al.*, 2000; Stringwell *et al.*, 2014; Le Vay *et al.*, 2007). In addition,

retaining individuals in production systems increases the costs and space requirements of culture (Obata *et al.*, 2008). Therefore, quantifying the trade-offs between larger sizes-at-release, maladaptation of juveniles to the wild and production costs are important for determining the optimal size-at-release, within the budget constraints of the release program.

In the early years of Penaeid shrimp (prawn) stock enhancement programs in China and Japan, early-life stage individuals were released, but these had little effect in enhancing stocks (Bell *et al.*, 2005). Later, the size-at-release was increased and juveniles were released at ~30 mm total length, with these individuals successfully contributing to increased fishery yields (Liu, 1990). The survival of the larger individuals was attributed to the shrimp's ability to escape short-term mortality through increased ability to adapt to the wild environment and escape predators (Bell *et al.*, 2005). The ~30 mm total length (~ 1 g wet weight), employed for shrimp release programs in China is similar to that determined for the Brown Tiger Prawn in the Exmouth Gulf (10 mm carapace length, ~40 mm total length and 1 g wet weight; Ye *et al.*, 2005). As malacostracans, such as prawns, increase in size, their abdomen increases in size and strength, which increases the strength of their "tail-flip". This mechanism is used as a startle response and while energetically expensive, it is an effective method to escape predators and respond to noxious environmental conditions (Edwards, 1995). Arnott *et al.* (1998) found that greater distance per tail-flip was achieved as the body length of the Brown Shrimp *Crangon crangon* increased. However, the greatest increases in both tail-flip distance and velocity were achieved during early development (*i.e.* > 6 mm body length onwards). Therefore, by releasing prawns at a size of ~30 mm, their ability to escape predation may be dramatically increased, leading to lower mortality rates.

Studies of the optimal size-at-release have also been conducted for the Blue Crab in Chesapeake Bay, USA (Johnson *et al.*, 2008). These studies found that the survival of juvenile crabs increased almost linearly from a carapace width of 20 to

50 mm before plateauing indicating that larger individuals attained a partial refuge from predation (Fig. 2.5). It is thought that larger crabs (i) may attain a size refuge from gape-limited predators (Hart and Hamrin, 1988), (ii) may be able to fight off predators (Hines and Ruiz, 1995) or (iii) may develop a method to escape predators (Christensen, 1996). While larger crabs survived better in the wild, smaller individuals were considered less likely to exhibit behavioural deficits caused by adaptation to the hatchery environment (Olla *et al.*, 1998), suffer reduced cannibalism during culture (Zmora *et al.*, 2005) and had lower costs of production than larger crabs. Therefore there was a trade-off between the increased post-release survival of larger crabs and the benefits of releasing smaller crabs. To overcome this, Johnson *et al.* (2008) found that if the crabs were released at a smaller size (< 40 mm carapace width [CW]) in spring, they grew rapidly to the size of > 40 mm CW, where mortality was lower (Fig. 2.5), therefore gaining the benefits of the smaller crabs, but growth was quick enough to offset a large proportion of the increase in predation due to the smaller size.

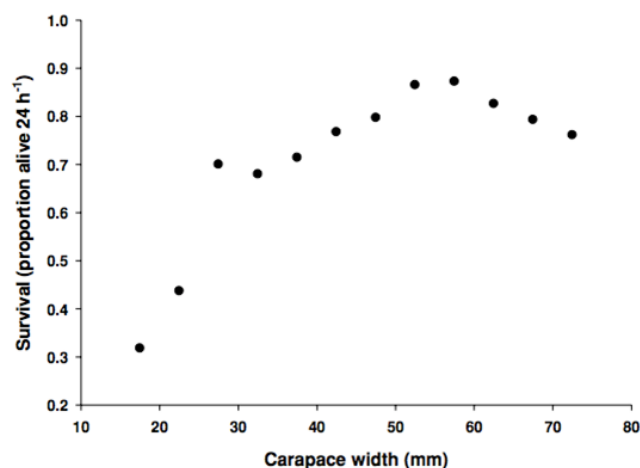


Figure 2.5: Mean (+ SE) survival of tethered Blue Crabs (*Callinectes sapidus*) of various sizes over a period of 24 h (Johnson *et al.*, 2008).

2.3.3: Time of release

All water bodies are subject to diurnal, seasonal and potentially tidal changes (*e.g.* Boyer *et al.*, 1999). As a result, the timing of the release of cultured

juveniles can have a direct and substantial impact on the success of enhancement (Leber *et al.*, 1997, 1998). Most species show a diurnal pattern of behaviour (*e.g.* Forward, 1976) and therefore when determining the time of day to release cultured juveniles, this pattern must be understood for both the species to be released and potential predators. For example, Mercier *et al.*, (1999, 2000) identified that juvenile Sandfish *Holothuria scabra* express a well-defined daily burrowing cycle; burrowing at sunrise for about 12 h and surfacing to feed at night. The fact that juveniles foraged at night was suggested as a protective measure from predators, a response found in a number of other echinoderm species (Nelson and Vance, 1979). Therefore, it was recommended that release of hatchery-reared juveniles occur at night or early morning to reduce the short-term mortality by predation on released juveniles.

A comparison of the diets of fish species present at the release site during the time when post-larval Western School Prawns *Metapenaeus dalli* were released indicated that the key predators were more abundant at night and that these fish fed on greater numbers of prawns during this time (Poh, B. Murdoch University, unpublished data), a trend consistent with that observed by Hoeksema and Potter (2006). Visual observations during releases of post-larval *M. dalli* (15 days from mysis) indicated that that during the day, they actively swam to the bottom, where they were camouflaged, whereas at night they drift passively in the water column (Poh, B. Murdoch University, unpublished data), thus exposing them to greater risk of predation. This data suggests that, any release program for the Western School Prawn should be conducted during the day, when the larvae will actively swim to the substratum and avoid immediate predation post-release.

Most ecosystems, particularly in temperate regions, exhibit seasonal changes in their environmental conditions, such as water quality, temperature or sediment composition (*e.g.* Boyer *et al.*, 1999), as well as changes in biotic factors such as their fish assemblages (*e.g.* Claridge *et al.*, 1986; Loneragan *et al.*, 1989; Tweedley *et al.*, 2016). These marked seasonal changes affect the growth and

survival of aquatic animals and are therefore likely to influence post-release survival. For example, Stoner and Glazer (1998) found that the survival rates of Queen Conch *Lobatus gigas* released in the Bahamas were higher during autumn, when water temperatures were lower. Cultured juvenile European Lobsters released in Norway suffered greater mortality in summer, due to predation by labrid fishes, which were more abundant at that time of year (van der Meeren, 2000). Similarly, the survival rates of restocked Blue Crabs in Chesapeake Bay appeared to be primarily driven by the seasonal change in abundance of predators. However, in this case, juvenile mortality was largely attributed to cannibalism by adult conspecifics, as peak mortality occurred in August, which coincides with the maximum abundances of adult Blue Crabs and the time when adults switch from feeding on infaunal bivalves to alternate prey items such as the juvenile crabs (Hines and Ruiz, 1995; Johnson *et al.*, 2008). It should also be noted that environmental conditions may influence the survival of Blue Crabs, with very low water temperatures during winter combined with low salinities causing mortality, particularly for juveniles (Rome *et al.*, 2005; Hines *et al.*, 2008).

In a stock enhancement program for Sea Mullet *Mugil cephalus* in Kaneohe Bay, Hawaii, cultured fish were released during different times of the year to evaluate the effect the release-time (summer and spring) had on survival, relative to wild juvenile recruitment (Fig. 2.6). The survival rates of fish below 70 mm TL and between 110-130 mm TL were both greater when released in spring, when juveniles recruit in the wild, than in summer. As a result it was determined that spring was the better season to release cultured Mullet in order to successfully enhance the commercial fishery's population (Leber and Arce, 1996).

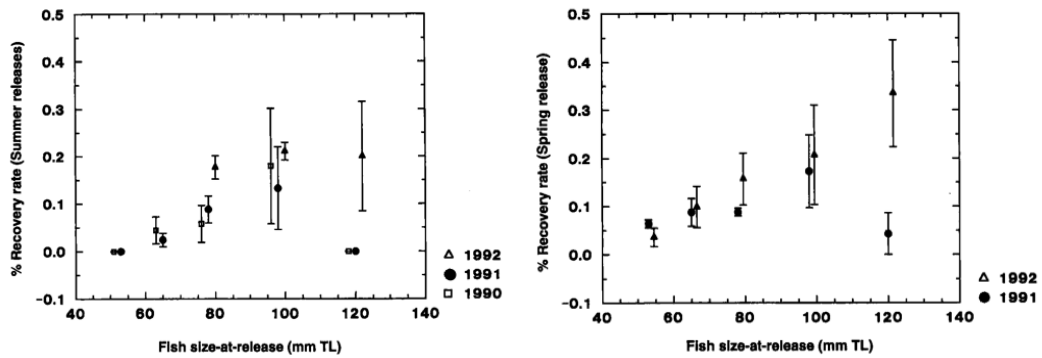


Figure 2.6: Mean percentage recovery rate of cultured Mullet (*Mugil cephalus*) in the commercial fishery following summer (left) and spring (right) releases into Kaneohe Bay, Hawaii. Taken from Leber and Arce (1996).

2.3.4: Genetic effects and broodstock collection

Concern surrounding the genetic effects of stock enhancement activities on natural populations is also common (Cross and King, 1983; Nielsen *et al.*, 1994). One of the major risks in release programs is to inadvertently reduce the genetic diversity of stocks through the mixing and dilution of wild stocks with genetically inferior hatchery-reared stocks (Frankham, 2005). This loss in genetic variation within the population predominantly arises from genetic drift due to utilising broodstock from too few individuals, or from interbreeding, whereby closely related individuals breed (Le Vay *et al.*, 2007). Thus before culturing, it is important to ensure that steps are taken to minimise loss of diversity and genetic alterations in the population. Le Vay (2007) proposed guidelines on broodstock collection and how to avoid this, recommending a minimum of 50 spawners, preferably with equal numbers of males and females to reduce loss of rare alleles, and to avoid bottleneck effects of already small populations. As well as ensuring that adults are collected for broodstock so that they constitute an unbiased sample of the naturally spawning donor population with respect to spawning timing, size, age, sex ratio and other traits important for long-term fitness of the stock.

2.4: Maximising social and economic benefits of release programs

Recreational fishing is a popular activity worldwide, with high social and economic values (Post *et al.*, 2002; Cooke and Cox, 2004; Allan *et al.*, 2005). Likewise commercial fisheries bring value to the community in the form of fresh fish sold locally to marketplaces, and directly to locals, tourists and visitors from nearby towns. In order to maintain these values, the production of fisheries must be sustained and this relies on the ability of aquatic ecosystems to support the production of fish (Post *et al.*, 2002) on top of the effective management of stocks. Stock enhancement is considered one of the better means of improving or maintaining the socio-economic values and objectives (*i.e.* market value from fishing trips and angler satisfaction) of a fishery without depleting the population (Camp *et al.*, 2013), while maintaining fishing activities and not limiting access.

2.4.1: Social benefits

Many fisheries rely on the stocking of hatchery-reared fish to sustain good quality fishing for species with some social or cultural value to recreational fishers (Ellison and Franzin, 1992; Cowx *et al.*, 1998; Kozfkay *et al.*, 2006). As a result, when selecting a species for release, the social value of particular species to the recreational fishing community should be taken into consideration in order to uphold the social values of that fishery. In the Blackwood Estuary, Western Australia, Black Bream *Acanthopagrus butcheri* is a prized species by recreational fishers (Taylor *et al.*, 2005a) and is also fished by one commercial operator (Potter *et al.*, 2008). As populations of Black Bream are essentially confined to their natal estuaries, they are particularly susceptible to fishing pressure, with the effects of over-fishing difficult to overcome naturally through immigration from other systems (Potter, *et al.*, 2008; Cottingham *et al.*, 2015). The significance of Black Bream to fishers, together with this species being native, easily cultured and completing its entire lifecycle within the estuary they are released in (thus contributing to future generations) made it an ideal candidate for restocking. A release program in this estuary was started in 2001-2002 to restock the

population of Black Bream, however, while the restocking has proved successful (Cottingham *et al.*, 2015), no studies of the effect on social or economic values have been conducted.

A restocking project in the Lake District region of British Columbia, Canada, a region that features ~13,000 lakes > five ha in surface area, aimed to enhance the popular recreational and sport fish species Rainbow Trout *Oncorhynchus mykiss* and Lake Trout *Salvelinus namaycush*. Social studies suggested that fishing effort was allocated across the Lake District based on two factors; fishing quality, and travel time, with high levels of fishing effort recorded around population centres (Hunt *et al.*, 2011; Post and Parkinson, 2012). These findings on the behaviour and preference of fishers enabled stocking efforts to be targeted toward lakes utilised more intensively by fishers in order to provide maximum benefits to the greatest number of recreational fishers.

When deciding on a species to be restocked it is important to consider the value of particular species to an area and/or to the fishing community. In the two examples above, release programs were undertaken to enhance stocks of a fishery that had existing social values to the community and/or commercial sector. However, release programs could also potentially create a fishery and the associated social values.

2.4.2: Economic benefits

Most economic evaluations of stock enhancement projects worldwide have focused on stocking fish for capture in commercial fisheries. However, numerous cost/benefit studies have indicated that releasing recreationally important species should increase the benefit of money invested (Rutledge, 1989; Rutledge *et al.*, 1990, 1991; Rimmer and Russell, 1998). The recreational fishing sector is important to the economy of Australia. In a nationwide survey on recreational fishing in 2001 ~ 20 % of the nation's population fished at least once a year, harvesting ~27,000 tonnes of finfish and providing ~\$1.8 billion AUD to the economy (Henry and Lyle, 2003), a monetary value similar in magnitude to that of

Australia's commercial fisheries at the time (ABARE, 2002). The economic contribution of recreational fishing in Western Australia alone has been reported to be in excess of \$570 million a year (Penn *et al.*, 2003). An estimated \$338 million of this value was spent on fishing-related equipment and activities by anglers over a 12-month period in 2000-2001, at an average cost of \$706 per angler (Henry and Lyle, 2003).

As mentioned above, fishing for Black Bream has clear social values for fisher, but it is also economically important for the local area (*e.g.* Henry and Lyle 2003; Ferguson and Ye, 2008). Black Bream in the Blackwood Estuary support two fishing tour operators, fishing competitions and also provides a source of income for the sole commercial fisherman who operates in this system (Department of Fisheries, 2004). The commercial fisher's catch is virtually all sold locally, including to restaurants, fish and chip shops and in direct sales to residents, tourists and inland visitors from nearby towns, with local suppliers indicating that the demand for local fresh fish in south-western Australia was increasing in 2003 (Department of Fisheries, 2004).

Restocking Black Bream in the Blackwood Estuary also provides benefits to nearby communities due to the flow-on effects of attracting greater numbers of people to fish the released Black Bream (Jenkins *et al.*, 2006). For example, the first survey of recreational fishing in the Blackwood River, conducted in 1974/1975 showed that recreational fishers undertook ~16,500 days of fishing effort per year, with Black Bream being the most common species kept (Caputi, 1976). It is likely that, with considerable increases in the State's population in the 31 years since that study, that the number of recreational fishers would have increased markedly. Increases in fishing activity generate increased sales for local fishing retail stores *e.g.* tackle and bait, and other businesses, *e.g.* for food, petrol and accommodation. Prior and Beckley (2007) determined that expenditures by boat and shore-based anglers on bait, tackle and capital equipment were considerable contributors to the economy of the Augusta-Margaret River area. They also found that the

estimated catch rate for recreational fishers was 1/3 of that reported 30 years previously by Caputi (1976), likely due to the decline in fish stocks.

2.5: Measuring success

Blankenship and Leber (1995) stated, “*One of the most critical components of any enhancement effort is the ability to quantify success or failure*”. They suggested that release strategies should be refined to maximise survival through pilot-scale releases. These pilot studies should have broad aims that evaluate whether stocked fish can survive and recruit to commercial or recreational catches. If these pilot studies are monitored effectively, operational procedures that optimise the survival and cost-benefit, as well as minimising any adverse ecological effects can be identified. The value of release trials has been clearly demonstrated in a number of studies across a range of species, including Sea Mullet in Hawaii (Leber *et al.*, 1996), Red Drum in Texas (McEachron *et al.*, 1998), Black Bream in south-western Australia (Taylor, 2005b; Potter *et al.*, 2008; Cottingham *et al.*, 2015) and Brown Tiger Prawns in north Western Australia (Loneragan *et al.*, 2004). The importance of monitoring is reflected in the fact that, in Western Australia, the government policy on stock enhancement and restocking requires release programs to be monitored and evaluated (Department of Fisheries, 2013).

A set of tools have been developed to assess quantitatively the characteristics of a fishery throughout a release program or post-pilot study including population assessment models, bio-economic evaluation, post-release growth and survival models and genetic diversity studies (Lorenzen *et al.*, 2010). This framework of assessment can be used to explore the costs and benefits of marine release programs, providing low cost tools to determine the success or likelihood of success and quantitatively gauge the true potential of enhancement in the initial program evaluation phase (Blankenship and Leber, 1995; Hilborn, 1999; Leber, 2002; Lorenzen, 2005). This framework can be separated into stages over a

release programs lifetime, the initial pre-release stage, during implementation and operation, and post-program review (see Loneragan *et al.*, 2004). The value of models during all phases of a release program has been identified by a number of authors (Blankenship and Leber, 1995; Rothilsberg *et al.*, 1999; Loneragan *et al.*, 2006; Lorenzen *et al.*, 2010).

2.5.1: Initial program evaluation

In the initial or feasibility stages, bioeconomic models are used to assess the potential of a program. For example, Ye *et al.* (2005) used a bioeconomic model when determining the economic viability, biological effectiveness and risk of a release program for the Brown Tiger Prawn in the Exmouth Gulf. The quantitative modelling approach was developed in consultation with scientists, industry representatives and fishery managers to mitigate the high costs of experiments and guide research and the release program (Loneragan *et al.*, 2004; Ye *et al.*, 2005). The bio-economic model encompassed all aspects of a release program, from the collection of brood stock and production of prawns in the hatchery, through to their culture and release into the Exmouth Gulf and their eventual capture in the fishery. This model allowed researchers to explore and quantify the effect of uncertainty for various release scenarios, uncertainties around parameters in the model such as mortality, growth and post-release survival (Ye *et al.*, 2005) and to determine the main factors affecting release success. These simulations identified that variation in post-release mortality and density-dependant mortality had the greatest impact on the estimated returns to the fishery from a release program.

The model was also used to estimate the scale of release required to increase annual catches by 100 tonnes, the objective identified for the commercial scale required by the M.G. Kailis Company in discussions with researchers and managers (Loneragan *et al.*, 2004). The estimated 21 million kg of prawns required to achieve this objective would have required a large investment in infrastructure to support the production of the released prawns in the region, as no prawn

aquaculture facilities were present. This factor, combined with the estimated 60% probability of making a profit enabled M.G. Kailis Company to make an informed decision not to proceed with a commercial scale release program (Ye *et al.*, 2005). This example demonstrates the importance of quantitative analysis early in the planning of a release program to establish the potential success of a program and evaluate the costs and benefits involved.

2.5.2: Implementation and operation

During the implementation and operation of pilot releases, quantitative models may be employed to analyse the dispersal, growth and survival of released individuals. This has been applied to the enhancement of White Seabass *Atractoscion nobilis*, a mobile, pelagic coastal species in California. Approximately 1.1 million hatchery-reared White Seabass were released between 1986 and 2006, tagged with coded wire implants to identify hatchery-reared fish in the catch and allow recapture rates to be evaluated (Hervas *et al.*, 2010). The recapture of these tagged fish was also used to estimate the dispersal of the release fish and found that 95% of fish were captured within 135 km of their release site. Furthermore, quantitative models were used to assess many of the key components of a release program and indicated that timing and method of release affected the short-term post-release mortality of the cultured fish and that fish released in spring had a much higher chance of survival than those released in winter. Hervas *et al.* (2010) also used the model to determine that fish acclimated to natural conditions in a net-pen prior to release had a higher rate of survival than those released without acclimation. Finally, it was determined that, whilst the long-term mortality of released White Seabass was relatively low (*i.e.* 34.1 fish/year⁻¹) it was roughly double the rate of that of the wild White Seabass population (*i.e.* 16.5 fish/year⁻¹).

Leber (1999) stated that, "To resolve the unanswered questions about stock enhancement, fishery scientists must now work closer with the fishery managers who implement stocking" and suggested that adaptive management in pilot releases should be applied in all release programs. Likewise, Lorenzen *et al.* (2010)

also suggested that adaptive management must be firmly established as part of the operational plan for release programs. Adaptive management enables evaluation of the performance of a release strategy and provides a means to resolve uncertainties, improve efficiency of release strategies, refine operational plans and realign the program with the goals of the enhancement (Lorenzen *et al.*, 2010). Such an approach was used in research conducted in Hawaii to evaluate the potential of stock enhancement of the Sea Mullet (Leber *et al.*, 1996), and this was successful in increasing the contribution to juvenile recruitment by 600% over three years. To use adaptive management, a moderate level of ongoing assessment in the implementation and operation stage is required (Blankenship and Leber, 1995; Lorenzen *et al.*, 2010). New opportunities for refining the release program are constantly being created and integrated into the management process, therefore, aquaculture facilities and release strategies should be designed in a way that can be adapted relatively easily when modifications to release programs are deemed necessary (Blankenship and Daniels, 2004).

2.5.3: Post-program review

Post-program review is also an important stage in determining the impact that a released hatchery-reared population has on the total population of a species. For example, the release of marked Black Bream in the Blackwood Estuary, enabled the biological performance of juveniles of the 2001 and 2002 cohort released to be compared with those of wild fish, as well as assess the contribution they made to the population (Potter *et al.*, 2008; Cottingham *et al.*, 2015). Blankenship and Leber (1995) as well as Lorenzen *et al.* (2010) stated that a program that cannot identify the released fish is unable to determine if it has succeeded or failed, therefore marking the released fish is important for the post-program review. Black Bream were marked by staining the juveniles otoliths through immersion in a solution of Alizarin Complexone (ALC) and/or tagged using external T-bar tags (Partridge *et al.*, 2009). Sampling took place over a period of 12 years post-release, with Cottingham *et al.* (2015) describing the

performance and contribution of restocked Black Bream to commercial catches and egg production. These data showed that the 2001 cohort, released at seven months and a size of ~60 mm TL, had a significantly lower survival rate than the 2002 cohort, released at four months and at a size of ~40 mm.

Data collected on the ALC marked fish also indicated that the restocking project had a notable positive impact on commercial catches from 2006 onwards, with their contribution to the commercial fishery increasing from 6% in 2005 to 74% in 2010 (Cottingham *et al.*, 2015). This contribution subsequently declined to 39% in 2012, and further to 10% in 2014, which was attributed to a very strong recruitment of the 2008 year class. In 2008, restocked Black Bream were estimated to have contributed ~55% of eggs produced to spawning by the total population in this system, suggesting that substantial numbers of this 2008 year class were derived from spawning restocked fish from the 2001/2002 cohort (Cottingham *et al.*, 2015). The elements of mark and recapture methods allowed the contribution of the fish to the wild population to be evaluated with the long-lasting, easily identifiable mark on the otolith, and the micro-tags used. However, commercial fishermen in the Blackwood Estuary have also noted that they have been able to identify cultured fish from wild fish based on scale patterns, which has subsequently been verified in a small sample of fish (Tweedley, J. Murdoch University, pers. comm. 2016). This ability provides potential for a very low cost 'marking' or 'tagging' for future research.

Whilst it was stated that the use of marking to estimate the contribution of restocked fish to the population is necessary to identify success in a release program, different release program studies have suggested otherwise. Commercial or recreational catch data and size information from the fishery has also been used to provide an indication of whether a release program is successful or failing to enhance the fishery population. For example, if after a program to release prawns into an estuary, prawns start being caught at different times of year to those normally recorded in the fishery, it can be assumed that the release program is

effective. In Rekawa, Sri Lanka, the Asian Tiger Prawn *Penaeus monodon*, was the subject of a release program to increase catches in the commercial fishery (Davenport *et al.*, 1999). Rekawa Lagoon has an artisanal fishery for penaeids, predominantly the Indian Prawn *Penaeus indicus*, which is fished between October and May and contributes ~93.6% of the catch, with the Asian Tiger Prawn contributing <1% of the total catch and only being recorded from March to early May, but being of higher market value. Two post-larval releases of the latter prawn were carried out in July 1996 (~55,000) and July 1997 (~70,000) and it was estimated that if the juveniles survived they would reach fishable size by September/October and not interfere with the dominant Indian Prawn fishery. No marking was deemed necessary, as no fishable wild Asian Tiger Prawn would be expected in the lagoon at this time of year. After releases, the Asian Tiger Prawn was recorded in the commercial catch as early as September and until January, with annual total catch of the prawn increasing by 1,400% and total catch of the penaid fishery increasing by 18% (Davenport *et al.*, 1999). The authors concluded that this increase in catch and change in the timing of the catch provided sufficient evidence that the releases had been a success.

2.6: Key Considerations for Designing an Optimal Release Program

The success of release programs relies on identifying optimal release locations and times for the target species, based on temporal and spatial changes in the values of variables deemed to effect post-release survival of the species. Through a review of past literature and undertaking pilot releases, these values can be determined for a particular species to be successfully released and an optimal release strategy can be designed. The key components that Blankenship and Leber (1995) and Lorenzen *et al.* (2010) identified in their papers on the responsible approach to stock enhancement have been considered and have been used to identify the following 11 components that I consider to be of importance when planning and implementing an optimal release program (Table 2.2).

Table 2.2: Eleven components to be considered when planning and implementing an optimal release program.

-
- (1) Prioritise and select target species for enhancement**
This should be based on social and/or economic value to a community or for the conservation of a depleted stock. It should involve the engagement of stakeholders to assist in the decision-making of which species is to be enhanced.☒
 - (2) Assess the social or economic benefits and costs of the release program**
Assess what benefits and costs are associated with the program socially (enhancement of a species that holds some social or cultural value to the community) and/or economically (enhancement of a species will provide some source of income for commercial fishers, the recreational fishing sector and/or local businesses, *e.g.* tackle shops, local food stores and petrol).
 - (3) Develop objectives and targets for the release program**
Determine quantitative objectives or targets for the release, so that post-program review of success can be quantified.
 - (4) Develop appropriate culturing facilities and techniques**
Optimal hatchery size, structure and techniques used for feeding and predator training should be identified prior to culture to ensure that optimal quality juveniles are being produced with the highest chance of survival in the wild.
 - (5) Select an adequate supply of spawners**
A minimum of 50 spawners is recommended, to minimise loss of diversity within the population, and so that genetic alterations remain.
 - (6) Ensure that cultured stocks can be identified**
Tagging of fish using methods such as otolith staining, external tags (*e.g.* t-bar tags) or some other mean should be undertaken so that identification of released juveniles can be undertaken, allowing for easy post-program review.
 - (7) Use Disease and Health Management**
A health management process such as health screening of cultured stock should be carried out prior to release to ensure that disease is not being carried from the hatchery into wild population. Populations.
 - (8) Develop an optimal release strategy**
This should include those of release site, time and size, to ensure that cultured juveniles have the best chance at survival in the wild. As well optimising survival during the transport to release site and actions at release.☒
 - (9) Undertake an effective monitoring program**
Post-release monitoring is vital in order to conduct assessments into the success of a release program.
 - (10) Assess and manage the released stock**☒
Empirical assessment of the data gathered during monitoring for determining the performance of the program and whether or not objectives and targets are being met.
 - (11) Use adaptive management**
Adaptive management enables evaluation of the assessment and provides means to alter the program if required in order to optimise success and refine operation so as to meet objectives and targets of the program.
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Chapter 3: Optimising the release of *Metapenaeus dalli* in the Swan-Canning Estuary using the Survival Maximisation At Release Tool (SMART)

3.0. Abstract

Chapter 2 identified the significance of developing sound release strategies to optimise the performance of aquaculture-based enhancements and that few quantitative tools have been developed to guide release programs. The aim of this chapter was to create a tool to inform the development of an optimal release strategy, by evaluating site selection and time of release for the release of post-larval Western School Prawns *Metapenaeus dalli* in the Swan-Canning Estuary. This was achieved by developing the Survival-Maximisation-at-Release-Tool (SMART), a quantitative tool that collates variables considered to affect the survival of released *M. dalli* at potential sites around the estuary and determines a SMART score (0-100) for each potential release site and time (Month, Year, Day/Night). The major factors incorporated in SMART were water quality (salinity and water temperature), sediment composition and densities of conspecifics (total *M. dalli* and gravid *M. dalli*), competitors (*P. latisulcatus*) and teleost predators. The scores for each factor were given equal weighting and the average of these used to calculate an overall SMART score for each site and time. Statistical analyses on the SMART scores determined that region of release was the most influential factor on the survival of released prawns, followed by year and then month, and that the salinity, sediment composition and predation variables had the most influence on overall SMART score. The optimal site of release identified was at Deep Water Point in the Lower Canning Estuary during the night in January 2014. Further enhancements to the SMART are identified and mechanisms for adapting this tool for its application to other species and/or ecosystems are discussed. An output of the SMART is also presented showing how the tool can be readily conveyed to diverse audiences to enhance discussions on optimal release strategies.

3.1: Introduction

The Western School Prawn *Metapenaeus dalli* is the only metapenaid found in temperate south-western Australia (Racek, 1957), with its distribution in Australia ranging from Darwin in the north to Cape Naturaliste in the south (Grey *et al.*, 1983). At latitudes north of 31 °S *M. dalli* resides in inshore marine waters, however, south of this point, it is only found in estuaries and completes its entire lifecycle within these systems (Potter *et al.*, 1986; 1989; 2015b). Historically, *M. dalli* along with the Western King Prawn *Penaeus latisulcatus* were the focus of a small commercial fishery in the Swan-Canning Estuary in south-western Australia. After landings peaked at 15 tonnes in 1959, the prawn fishery declined through the 1960s until it closed, due to low catches, in the mid-1970s (Smith, 2006). *Metapenaeus dalli* was also the focus of a large and culturally significant recreational fishery, involving, at its peak, > 50,000 fishers from the Perth metropolitan area (Smithwick *et al.*, 2011).

After the cessation of commercial fishing, recreational catches still declined markedly, with the last significant catches recorded in the late 1990s, leading Maher (2002) and Smith (2006) to suggest that the *M. dalli* population was depleted and not yet recovered. Although the cause of decline is unclear, it was attributed to a combination of overfishing, changing environmental conditions and successive recruitment failures (Smith, 2006; Smith *et al.*, 2007). Thus, given the long-term inability to recover, a restocking program was seen as the best possible means of increasing the population of *M. dalli* in the Swan-Canning Estuary by circumventing the recruitment bottleneck during the high mortality stages from larval to juvenile prawns (Smith *et al.*, 2007). Such a strategy was very successful in rebuilding the Black Bream *Acanthopagrus butcheri* population in the Blackwood River Estuary, 300 km south of the Swan-Canning Estuary, with restocked fish contributing as much as 80% of the commercial catches in some years (Potter *et al.*, 2008; Gardner *et al.*, 2013; Cottingham *et al.*, 2015).

A trial restocking program for *M. dalli* was initiated in 2013, releasing over 4.5 million hatchery reared post-larval prawns (PL10-15, *i.e.* 10-15 day old post-larvae) at various locations in the shallow waters of the Swan-Canning Estuary from late October 2013 until March 2016. These sites were selected by scientists/environmental managers and keen recreational fishers based on expert judgment to be the best suited for the prawns. A scientific program investigating the biology and ecology of *M. dalli* in the Swan-Canning Estuary was run parallel to the restocking project and produced, for example, information on the effects of salinity and water temperature on larval growth and survival (Crisp *et al.*, 2017), sediment preference (Bennett, 2014), growth and reproduction (Broadley *et al.* 2017), spatial and temporal patterns of abundance and identification of teleost species that predate on the released juvenile prawns (Poh, B., Murdoch University, unpublished data). Rather than relying on expert judgement, the recent availability of these data now enable the development of an objective, quantitative approach to determine the optimal release site(s) for hatchery reared post-larval *M. dalli*.

Despite an extensive search of the literature (see Chapter 2), only a single study was identified that had attempted to use an objective, quantitative approach for selecting release sites for individuals. In this study, Carvalho and Gomes (2003) developed a tool to identify the optimal release site for the European Wild Rabbit *Oryctolagus cuniculus*, a species of conservation significance on the Iberian Peninsula, based predominantly on the attributes of habitat type, *i.e.* tall scrub, rocky terrain, grassland. The lack of similar studies is surprising as there has been an increase in the popularity of release programs around the world over the last thirty years (Taylor *et al.*, 2016). Given (i) the considerable financial costs in producing hatchery-reared individuals, (ii) the fact that many release programs are not successful (Bell *et al.*, 2005; Lorenzen, 2005) and (iii) the lack of tools to aid in the selection of release sites and times, there is a need to develop a tool to enable the quantitative selection of appropriate release sites and times to maximise the survival of hatchery-reared individuals.

In light of the above, the broad aim of this chapter is to develop the Survival-Maximisation-At-Release-Tool (SMART), a quantitative tool to aid in the selection of the most appropriate release sites and times for hatchery-reared *M. dalli* in the Swan-Canning Estuary. In order to achieve this, the specific aims of this Chapter are to:

1. Determine those factors associated with release sites that are likely to affect the survival of released *M. dalli* (Section 3.2.2).
2. Determine the spatial and temporal variation in these factors within the Swan-Canning Estuary (Section 3.3.1).
3. Develop an approach for scaling the values for these factors at each site onto a common scale for inclusion into the final model (Section 3.2.4).
4. Critically review the output of the model and its effectiveness in selecting a release site, as well as identifying improvements that could be made to the model and its underlying data layers (Section 3.4).

Note that the main focus of this chapter is evaluating the selection of sites for night releases, as most of the underlying data was collected during a monitoring program for *M. dalli* that was undertaken at night. However, as a preliminary investigation of the magnitude of predation on *M. dalli* by teleosts suggested that day time releases would increase survival (B. Poh, Murdoch University, unpublished data) and information on the composition of the fish faunas at a similar range of sites during the day at the same time of year was readily available from the Fish Community Index project (Hallet and Tweedley, 2014; 2015; 2016), the SMART was also employed to investigate, in brief, the best release sites for day-time release and day vs night releases at the same site.

3.2: Materials and methods

3.2.1: Site description

The Swan-Canning Estuary is a shallow, permanently open system located in the Perth metropolitan region of south-western Australia (Fig. 3.1). The estuary is ~50 km long, covers ~55 km² in area and comprises of a narrow entrance channel, two basins (Melville and Perth Waters) and the tidal portions of the Swan and Canning Rivers (Valesini *et al.*, 2014). The majority of the estuary is shallow, *i.e.* < 2 m in depth, with extensive sand flats of ~0.5 m deep, however, it reaches a maximum depth of ~20 m in the entrance channel. Estuaries in south-western Australia are microtidal (tidal range <2 m) and experience a typical Mediterranean climate, with hot, dry summers and cooler, wet winters (Gentili, 1971), leading to pronounced seasonal variations in environmental conditions in the estuary (Tweedley *et al.* 2016a).

The estuary flows through the greater Perth metropolitan area, which supports ~78% of the 2.6 million people in the state of Western Australia (Australian Bureau of Statistics, 2015). The system has been extensively modified by anthropogenic activity (National Land and Water Resources Audit, 2002a, b) and, as a result, has led to multiple stressors on the system, such as increased delivery of sediments and nutrients, in addition to changes in salinity and hydrological regime, including periodic hypoxia (Stephens and Imberger, 1996; Tweedley *et al.*, 2016b). Despite these perturbations, the estuary is highly valued for its aesthetic, cultural and social significance to residents and tourists of the area and recreational fisheries (Malseed and Sumner, 2001).

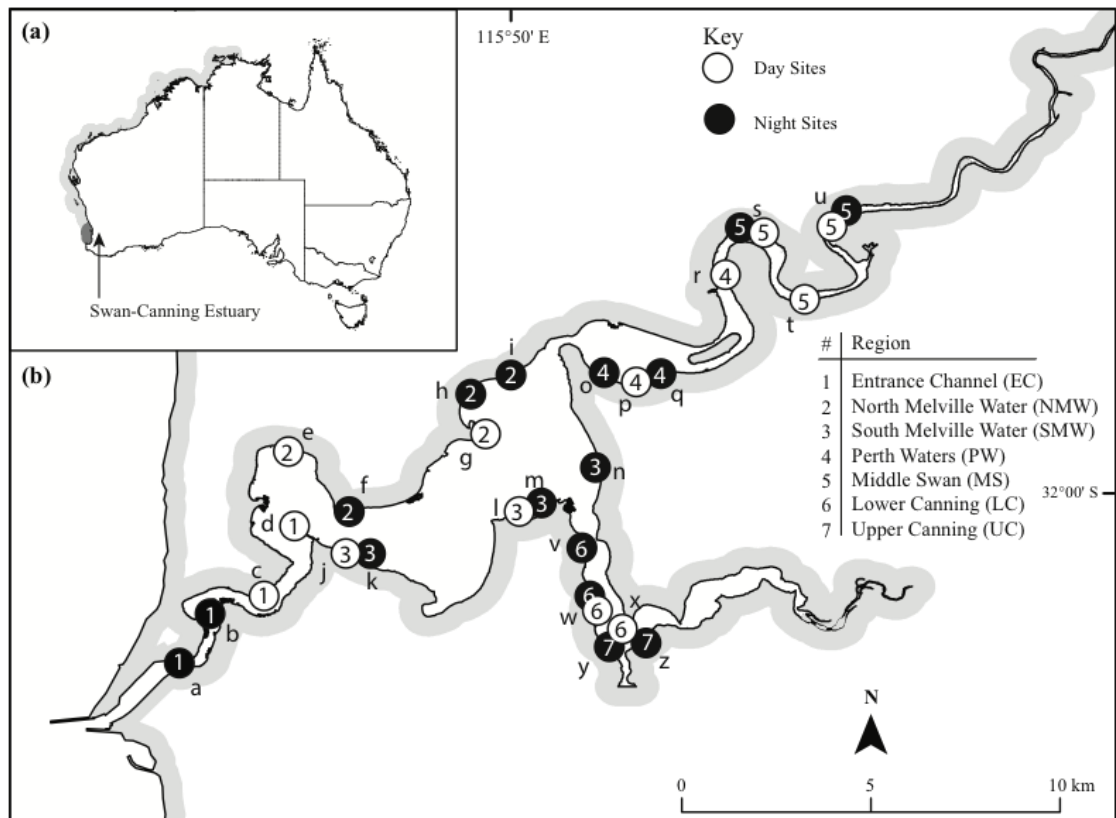


Figure 3.1: Map showing (a) Australia and the distribution of *Metapenaeus dalli* in inshore marine waters (light grey) and solely in estuaries (dark grey) and the location of the Swan-Canning Estuary in south-western Australia and (b) location of the sites sampled during the day (○) and night (●). The number inscribed in each site symbol denotes the region of the estuary that sites belongs to. Sites and abbreviations used later are as follows; a = Stirling Bridge (SB), b = Leeuwin Barracks (LB), c = Chidley Point (CP), d = Point Walter (PTW), e = Claremont (C), f = Dalkeith (DK), g = Pelican Point (PP), h = Matilda Bay (MB), i = Kings Park (KP), j = Attadale (A), k = Point Walter (PTW), l = Heathcoate (H), m = Applecross (A), n = Como (CO), o = South Perth (SP), p = Perth Water (PW), q = Coode St. (CS) r = Windan Bridge (W), s = Maylands, (ML) t = Belmont (B), u = Garratt Rd. Bridge (GRB), v = Canning Bridge (CB), w = Deep Water Point (DWP), x = Mount Henry Bridge (FW), y = Freeway (FW), z = Rossmoyne. (R).

3.2.2: Rationale for selecting variables

To minimise the number of variables, only those that were deemed to be influential (positive or negative), on the survival of hatchery-reared post-larval *M. dalli*, were incorporated into SMART. The following section describes the rationale for the choice of variables included in the model, which were selected in conjunction with stakeholders and experts including the Department of Parks and Wildlife, Department of Fisheries and the Australian Centre for Applied Aquaculture Research at South Metropolitan TAFE.

Water quality

Laboratory experiments investigating the influence of salinity and water temperature on the survival of cultured *M. dalli* demonstrated that both variables have a significant effect on the survival of the protozoal and mysis stages of *M. dalli* (Crisp *et al.*, 2017). Whilst this experiment was conducted using larval stages, the results are likely to also apply, to some extent, to the post-larvae. In addition to direct effects on survival, adverse salinities and water temperatures will slow growth due to increased energetic cost of homeostasis, and also impair foraging, slow movement and therefore increase the risk of predation.

In addition, due to changes in the volume of freshwater discharge and air temperature, salinity and water temperature vary spatially throughout the Swan-Canning Estuary and temporally throughout the year (*e.g.* Hoeksema and Potter, 2006; Broadley *et al.*, 2017). Fauna living within the system, thus respond to these changes. For example, the growth of *M. dalli* is much faster when water temperatures were $>20^{\circ}\text{C}$, which was also the time when female prawns started to become gravid and spawning occurs (Broadley *et al.*, 2017). As a result, it is important to account for spatial and temporal changes in water physico-chemistry within SMART and score positively sites that have optimal salinities and water temperatures to promote survival of hatchery-reared post-larval *M. dalli*.

Note that dissolved oxygen concentration was recorded during the major sampling program along with salinity and water temperature (see later) and that crustaceans are particularly sensitive to this physico-chemical variable (Wu *et al.*, 2002; Dauvin and Ruellet, 2007; Tweedley *et al.*, 2016b). However, while this variable was considered influential for the survival of hatchery-reared post-larval *M. dalli*, no major differences in dissolved oxygen concentration were detected among spatially or temporally and no hypoxic events were recorded in the shallow waters. This variable was therefore excluded from the model.

Sediment composition

The proportion of particulate organic matter and inorganic grain sizes were found to affect the distribution and abundance of *M. dalli* in the Swan-Canning Estuary during summer, *i.e.* the time when hatchery-reared individuals would be released (Bennett, 2014). Moreover, laboratory experiments demonstrated that this species preferred sediments with a higher proportion of finer and/or lower portion of larger grain sizes and that prawns were better able to bury rapidly in finer sediment, thus reducing their exposure to predators (Bennett, 2014). Given their strong association with the benthos, it is not surprising that, in addition to *M. dalli*, other penaeid species have also been shown to exhibit a preference for particular sediments (*e.g.* Hughes, 1966; Branford, 1980; Somers, 1987).

Abundance of competitors

Competition among species occurs on one or more of three axes, *i.e.* food, space and time (Ross, 1986). Thus, competition for food and shelter may negatively affect the post-release survival of cultured individuals (*e.g.* Tremblay, 1991; Støttrup and Sparrevohn, 2007; Ochwada-Doyle *et al.*, 2012). For example, it was observed that an increase in weight and yield of Brook Trout *Salvelinus fontinalis* was inversely correlated with the occurrence of White Sucker *Catostomus commersonii*, attributed to the competition caused by a strong dietary overlap between these two species (Tremblay, 1991). Along with *M. dalli*, the Western King Prawn (*Penaeus latisulcatus*) and Linda's Velvet Prawn (*Metapenaeopsis lindae*) are also found in the shallow waters of the Swan-Canning Estuary (Manning, 1988; Potter *et al.*, 1991). Due to the similar morphology and likely also the diets of these species, it is possible these other species may compete for resources with the hatchery-reared *M. dalli* and lower their survival. While densities of up to 12 individuals 500 m⁻² of *P. latisulcatus* were recorded at some sites over the study period, only low numbers of *M. lindae* (*i.e.* up to 1 individuals 500 m⁻² and < 1% of samples) were recorded and thus the density of the latter competitor species was excluded as a variable from the model.

Abundance of conspecifics

A study on the hatching and survival of decapod species concluded that each species exhibits a preference for a particular range of salinities and water temperatures and that above or below these conditions larval mortality increased (Roberts, 1971). Likewise, Preston (1985) demonstrated that the larval survival of the Greentail Prawn (*Metapenaeus bennettiae*) was greatest when the larvae were raised in conditions that mimicked the water temperature and salinity conditions present at the time of spawning. Both the total density of *M. dalli* (male and females) and the density of gravid females were considered a positive influence for predicting the survival of released post-larvae. This was because a higher density of gravid female *M. dalli* was thought to indicate the location of a preferred spawning ground and the preferred time of year for spawning in the natural population, thus one with optimal conditions for the hatching and survival of larvae and likely also juvenile *M. dalli*.

Predation by teleost and scyphozoan species

Predation by fish species has been identified as potentially the single greatest hurdle in short-term post-release survival (Hines *et al.*, 2008; Støttrup *et al.*, 2008). For example, Buckmeir *et al.* (2005) found that ~27.5% of released Largemouth Bass (*Micropterus salmoides*) were lost to predation after just 12 hours and Dall (1990) suggested that 25% of juvenile prawns in coastal inland waters are lost each week, mainly due to predation. Stomach content analysis of teleost species collected from the site of a release of hatchery-reared post-larval *M. dalli* (Poh, B., Murdoch University, unpublished data), along with previous published studies of the diet of fish in the Swan-Canning Estuary, identified 19 species that may predate on hatchery-reared post-larval *M. dalli* (described in more detail below). In order to minimise the threat that these species pose on the survival of released *M. dalli*, the effect of predation from teleost species was included in the model to assist in selecting a site where their abundance and thus the influence of predation would be least.

When scyphozoans occur in high numbers, they collectively have a large clearance rates (i.e. the rate at which food particles can be ingested from the water column), significantly affecting the population size of zooplankton organisms (Hansson *et al.*, 2005; Hosia *et al.*, 2012). They have even been implicated in the collapse of fisheries, due to killing the larval stages (Hansson *et al.*, 2005). As a result, the abundance of local scyphozoan species was included in the preliminary analysis of the abundance and magnitude of predators.

3.2.3: Sources of data

Metapenaeus dalli exhibit a strong seasonal cycle of reproduction in the Swan-Canning Estuary, with gravid females first appearing in the shallow, nearshore waters in November and disappearing by late March (Broadley *et al.*, 2017). A similar pattern of seasonality was also recorded in a separate study, in the same estuary, from 1977-1982, with gravid *M. dalli* recorded between November and early April (Potter *et al.*, 1986). Current aquaculture practices for *M. dalli* require heavily gravid females to be captured from the wild (i.e. broodstock collection), rather than conditioning broodstock populations held in the hatchery (Jenkins *et al.*, 2015). Thus, the production of *M. dalli* in the hatchery is limited by the timing and duration of natural spawning. Given that larval development lasts for ~12 days and the post-larvae are being released 10-15 days after they metamorphose into post-larvae (Crisp *et al.*, 2016a,b), there is a one month lag between broodstock collected and the subsequent release of post-larvae. Although, some of the data used below were collected monthly over 36 months, only data from November to March in each of 2013/14, 2014/15 and 2015/16 were included in the model as this coincides with the production and release of hatchery-reared *M. dalli* (see Appendices Fig. 3.1).

While the original objective of this study was to create a tool to assist in selecting the optimal site for the release of *M. dalli* at night, data collected during the day were available at a similar range of sites. These data were subjected to the

tool, to determine the best release sites for day releases over each of three years and to also enable a day vs night comparison of a number of sites and months where data were available.

Water quality

Measurements of water physico-chemistry (*i.e.* salinity, water temperature and dissolved oxygen concentration) were recorded during the night at sixteen sites in the nearshore (< 1.5 m deep) waters of the Swan-Canning Estuary (Fig. 3.1), ranging from the Stirling Bridge, in the entrance channel, as far up the Swan River as Garratt Road Bridge and as far up the Canning River as Rossmoyne. Sampling was conducted every 28 days on a new moon on 31 occasions between October 2013 and March 2016. At each site, on each sampling occasion, salinity, water temperature and dissolved oxygen concentration were recorded at a depth of 1 m using a Yellow Spring International 556 Handheld Multiparameter Instrument.

Measurement of the same suite of water physico-chemical variables were recorded using the same equipment, only during the day, at 13 sites covering a similar proportion of the nearshore waters of the Swan-Canning Estuary (Fig. 3.1). Sampling was conducted in late January/early February in each of 2014, 2015 and 2016. Full details of the sampling methodology can be found in Hallett and Tweedley (2014; 2015) and Hallett (2016).

Sediment composition

Two replicate sediment samples were collected from each of the 16 sites sampled during the night (Fig. 3.1) on a single occasion during the month of February 2014. Full details of the sampling and laboratory protocols are described in Bennett (2014), but a brief summary of the methodological approach is provided here.

Samples were collected using a cylindrical corer, which was 3.57 cm in diameter and sampled to a depth of 10 cm. In the laboratory, the percentage contribution of particulate organic matter (POM) was calculated using the Loss of

Ignition method (Heiri *et al.*, 2001), and converted to a percentage (Hourston *et al.*, 2009). Fine sediment (*i.e.* particles <63 µm in diameter) was removed from the inorganic portion of the sediment by wet-sieving through a 63 µm sieve before drying and re-weighing. Finally, the remaining sediment was wet-sieved through six mesh sizes corresponding to the Wentworth Scale for grain size, *i.e.* 63, 125, 250, 500, 1000 and 2000 µm (Wentworth, 1992). The inorganic fraction for each grain size was then dried, weighed and their percentage contribution by weight determined for each sample.

While the current study only utilised the nearshore sediment composition data from February 2014 (summer), Bennett, (2014) also collected sediment from the same sites in August 2014 (winter) and found that there was no statistical difference in composition. Given this lack of temporal changes in sediment composition in the nearshore waters, the data from summer 2014 was used throughout the model, *i.e.* in each month between November and March in 2013/14, 2014/15 and 2015/16. Although sediment composition was not expected to undergo a diel change, not all the day sites matched those sampled at night and thus the quantitative sediment maps produced by Bennett (2014) were used to estimate the sediment composition at sites where no empirical data were available.

Abundance of penaeids, teleosts and scyphozoans

Faunal sampling at each of the 16 sites sampled during the night (Fig. 3.1) occurred on each of the 31 sampling occasions (see above) and was conducted using a hand trawl net that was 4 m in width and constructed from 9 mm mesh. Although the net was 4 m wide when fully stretched, its 'functional' width when trawling was, on average, 2.85 m and thus, when dragged for 200 m, covered an area of ~570 m². Two replicate trawls were conducted at each site, on each sampling occasion.

Upon completion of the drag, the contents of the net were emptied and the fauna identified and enumerated. Penaeids were immediately identified to species,

sexed (*i.e.* females identified by presence of a thelycum and males by the presence of a petasma) and counted. In addition to being counted as part of the total number of *M. dalli*, gravid female prawns that were readily identified macroscopically by the appearance of a distinct green gonad (Crisp *et al.* 2016b) were also counted separately. After processing, all penaeids were returned alive to the water. The number of individuals of each teleost and scyphozoan species recorded, except in the case of the Spotted Hardyhead (*Craterocephalus mugiloides*), Elongate Hardyhead (*Atherinosoma elongata*) and Presbyter's Hardyhead (*Leptatherina presbyteroides*), which were grouped together as 'Athernidae' as, due to their large abundances and similar morphology could not be identified quickly enough at night in the field to enable them to be returned to the water alive. As with any penaeids, all teleosts and scyphozoans were returned to the water alive as per the instructions in Murdoch University Animal Ethics Committee permit #RW2566.

The abundance of each teleost species was quantified, during the day, at 13 sites in the Swan-Canning Estuary during late January/early February of 2014, 2015 and 2016 (Fig. 3.1) as part of sampling for the Fish Community Index for the estuary (Hallett and Tweedley 2014; 2015; Hallett, 2016). In those studies, one replicate sample was collected at each site, on each sampling occasion, using a seine net. The net was 1.5 m in height, 21.5 m in length, with two 10 m wings constructed from 9 mm mesh and 1.5 m wide bunt comprising 3 mm mesh and covered an area of ~116 m²). Once a sample had been collected, large and easily identified teleost species were counted and returned to the water alive, while smaller fish were euthanised in an ice slurry and subsequently identified in the laboratory. Note that all teleost individuals were identified to species and recorded, however, information on the abundance of penaeids and scyphozoans was not recorded. Full details of sampling are provided in Hallett and Tweedley (2014; 2015) and Hallett (2016) and covered under Murdoch University Animal Ethic Committee permits RW2464 and RW2706.

While no data are available on the abundance of penaeids at sites sampled during the day, many studies of the activity patterns of juvenile penaeids and metapenaeids have recorded a strong diel pattern of burrowing (*e.g.* Wickham and Minkler, 1975; Hill, 1985; Dall *et al.*, 1990; Wassenberg and Hill, 1994; Honculada Primavera and Lebata 1995). These demonstrate the penaeids burrow into the substrate at sunrise, remain inactive in the sediment throughout the day, and emerge again at dusk, remaining active above the substrate throughout the night. Similarly, Bennett (2014) observed that *M. dalli* held in the laboratory actively foraged above the sediment during the darkness, before burying into the sediment and remaining inactive during daylight. Thus the abundance of each penaeid species has been assigned a score of 0 in each replicate sample collected during the day.

3.2.4. Scaling of data and inclusion in SMART

The aims of SMART are to objectively combine those variables, *i.e.* salinity, water temperature, sediment composition and the abundance of penaeids, teleosts and scyphozoans, that are thought to influence the survival of hatchery-reared post-larval *M. dalli* and thus should be considered in the selection of a suitable release site within the Swan-Canning Estuary. As many of these variables are measured on different scales, standardisation is needed to place all data on a common scale and thus allow comparability.

The first part of this section will explain how the data for each variable was standardised, with the second focusing on the combination of the variables and development of the SMART model. Focus is mainly placed on the production of SMART for data collected at night, as this was the original aim of the Thesis, and the diel period with the most comprehensive data. However, the changes in the development of the day and/or day vs night models will be highlighted.

3.2.4.1: Data standardisation

This section details the steps taken to standardise each of the variables included in SMART (Table 3.1). These steps are simplified in a flow chart below

(Fig. 3.2), with additional detail provided in the text. The second figure (Fig. 3.3) indicates those values that were included in the tool and those that were removed after being deemed not to have a large enough effect on the post-release survival of *M. dalli* as explained in the text.

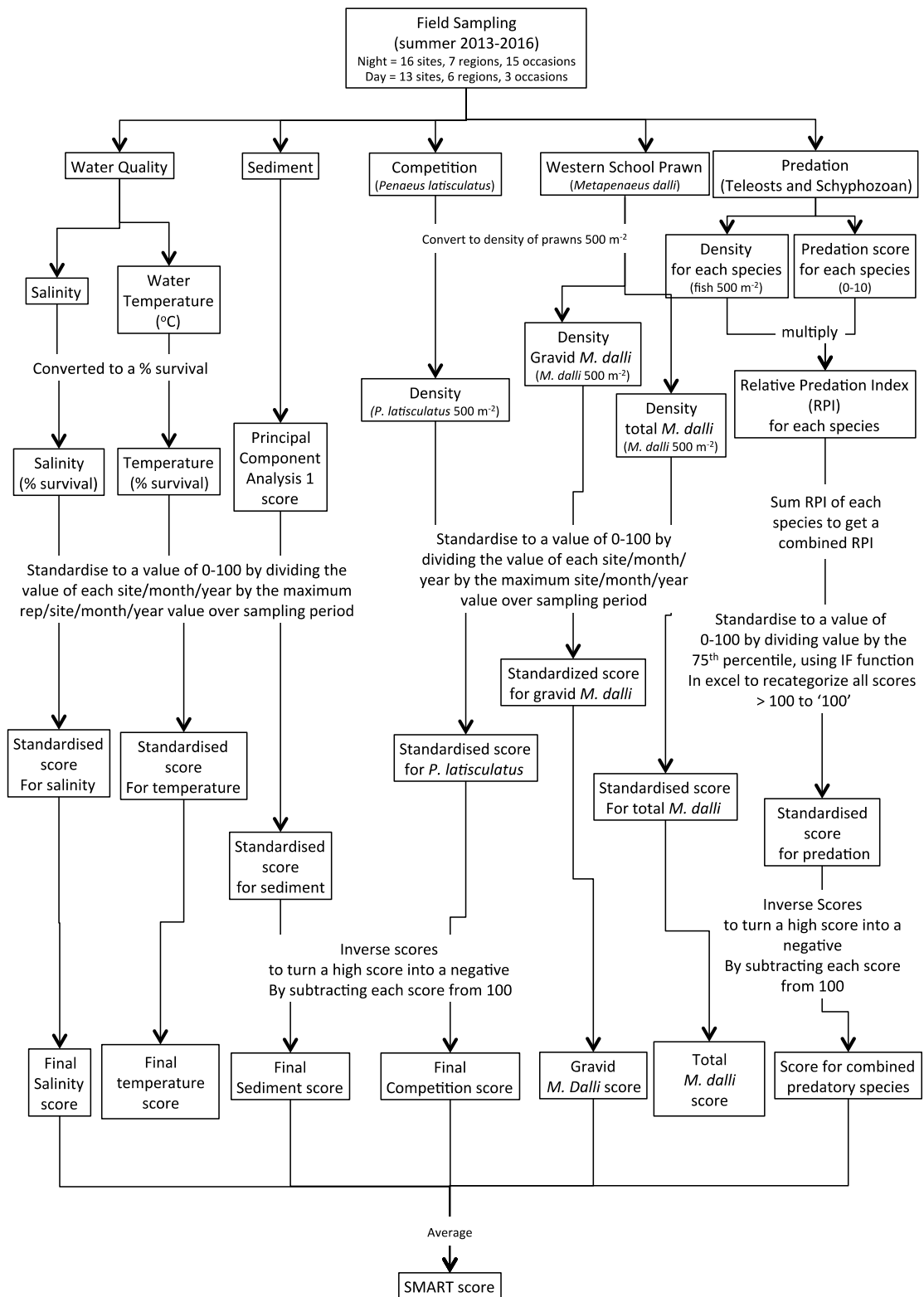


Figure 3.2: Flowchart outlining all of the data and process used to produce SMART.

Table 3.1: Factors and Variables considered for inclusion in the SMART.

Factors and Variables				
Category	Variable	Units	Hypothesised Pos/Neg Effect	Included
Water quality	Water temperature	°C	+/-	Y
	Salinity	‰	+/-	Y
	Dissolved oxygen concentration	mg/L	+/-	N
Sediment	Sediment	PC1 Score	+/-	Y
Competitors	<i>P. latisulcatus</i>	Density (500m ⁻²)	-	Y
Conspecifics	Total <i>M. dalli</i>	Density (500m ⁻²)	+	Y
	Gravid female <i>M. dalli</i>	Density (500m ⁻²)	+	Y
Predators	<i>A. elongata</i>	Density (100m ⁻²)	-	Y
	<i>C. mugiloides</i>	Density (100m ⁻²)	-	Y
	<i>L. presbyteroides</i>	Density (100m ⁻²)	-	Y
	<i>O. rueppellii</i>	Density (100m ⁻²)	-	Y
	<i>A. vaigiensis</i>	Density (100m ⁻²)	-	Y
	<i>F. punctatus</i>	Density (100m ⁻²)	-	Y
	<i>A. butcheri</i>	Density (100m ⁻²)	-	Y
	<i>L. wallacei</i>	Density (100m ⁻²)	-	N
	<i>P. olorom</i>	Density (100m ⁻²)	-	N
	<i>E. australis</i>	Density (100m ⁻²)	-	N
	<i>A. caudivittata</i>	Density (100m ⁻²)	-	N
	<i>T. pleurogramma</i>	Density (100m ⁻²)	-	N
	<i>G. holbrookii</i>	Density (100m ⁻²)	-	N
	<i>P. octolineatus</i>	Density (100m ⁻²)	-	N
	<i>A. suppositus</i>	Density (100m ⁻²)	-	N
	<i>S. burrus</i>	Density (100m ⁻²)	-	N
	<i>H. semifasciata</i>	Density (100m ⁻²)	-	N
	<i>R. sarba</i>	Density (100m ⁻²)	-	N
	<i>U. carinirostrus</i>	Density (100m ⁻²)	-	N
	<i>E. machnata</i>	Density (100m ⁻²)	-	N
	<i>H. vittatus</i>	Density (100m ⁻²)	-	N
	<i>S. schomburgkii</i>	Density (00m ⁻²)	-	N
	<i>P. jenynsii</i>	Density (100m ⁻²)	-	N
	<i>S. nigra</i>	Density (100m ⁻²)	-	N
	<i>S. robustis</i>	Density (100m ⁻²)	-	N
	<i>M. cephalus</i>	Density (100m ⁻²)	-	N
	<i>H. melanochir</i>	Density (100m ⁻²)	-	N
	<i>G. subfasciatus</i>	Density (100m ⁻²)	-	N
	<i>S. maculata</i>	Density (100m ⁻²)	-	N
	<i>P. punctata</i>	Density (100m ⁻²)	-	N
<i>A. aurata</i>	Density (100m ⁻²)	-	N	

Water quality

Salinity

Controlled laboratory experiments demonstrated that salinity significantly influenced the survival of larval *M. dalli* (Crisp *et al.* 2017). Larval *M. dalli* are likely to be more susceptible to the adverse effects of water physico-chemical conditions than the post-larval prawns that would be released (Pechenik, 1999), however similar studies on post-larvae *M. dalli* have not been conducted. While hatchery-reared *M. dalli* may be more resistant to variable salinity, indirect consequences of salinity, such as slow growth due to the metabolic costs of osmoregulation,

inability to forage efficiently or become slower moving would still result in more exposure to predation and thus increased mortality. The study by Crisp *et al.* (2017) demonstrated that a salinity of ~35 was optimal for larval *M. dalli*, with both lower (30) and higher (40) salinities decreasing survival (Fig. 3.3).

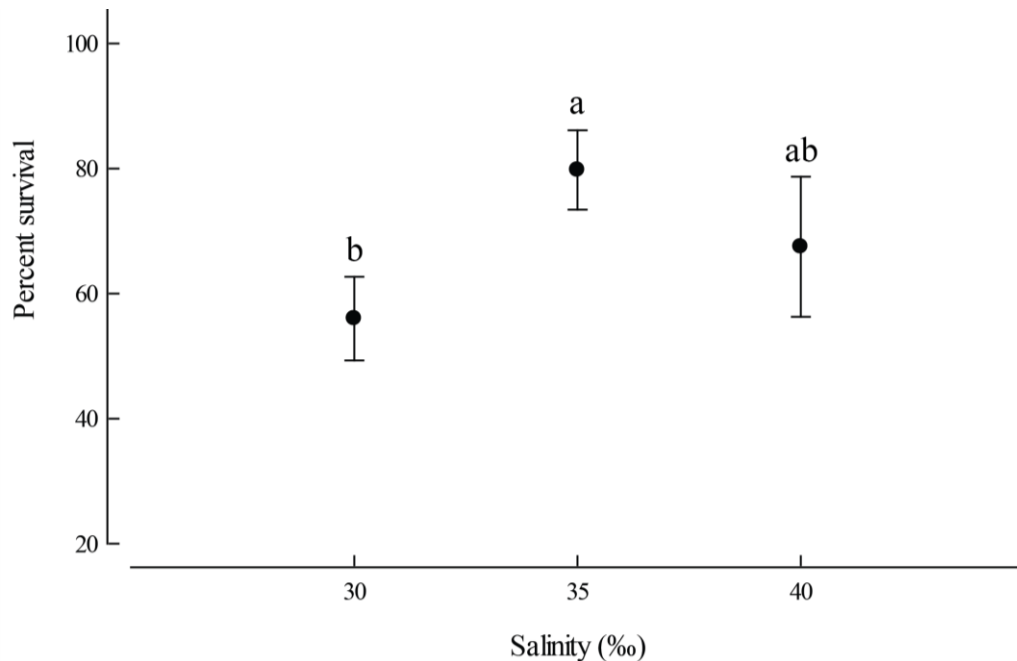


Figure 3.3: Mean percentage survival ($\pm 95\%$ CL) of *Metapenaeus dalli* larvae over a 48 h period from N VI sub-stage at three different salinities. Letters above error bars denote groups of samples identified by Tukey's HSD ($p < 0.05$). Taken from Crisp *et al.* (2017).

These data were used to estimate the influence salinity recorded at each site, on every sampling occasion, had on *M. dalli* percentage survival. This was done by extrapolating the results from Crisp (2017) to determine a percentage survival of *M.dalli* in the salinity at each site based on the salinity recorded at that site. To assign a percentage survival for salinities outside of those experimented by Crisp (2017) a linear line was followed to extrapolate, whilst this is likely not an accurate representation of the actual percentage survival it was only a small amount of the data that was outside of the known salinity percentage survival rates (~ 17% when November of 2013 is not included). These percentage values were then standardised onto the common scale of 0-100 by dividing each value by the maximum percentage survival and multiplying by 100. This formula produced the final score for salinity used in the model.

Water temperature

Similarly to salinity, controlled laboratory experiments undertaken by Crisp *et al.* (2017) showed that temperature significantly influenced the percentage survival of larval *M. dalli*. Survival rates were greatest at a temperature of 25.8 °C, with lower (22.6 °C) and slightly higher temperatures (29.4 °C), lowering survival albeit not significantly, while considerably higher temperature (32.6 °C) markedly reduced survival (Fig. 3.6). Data from this study was used in an identical manner to that in salinity to convert temperature recorded for each site into an expected percentage survival of *M. dalli*, which was then standardised. This standardised value was that representing temperature in the model.

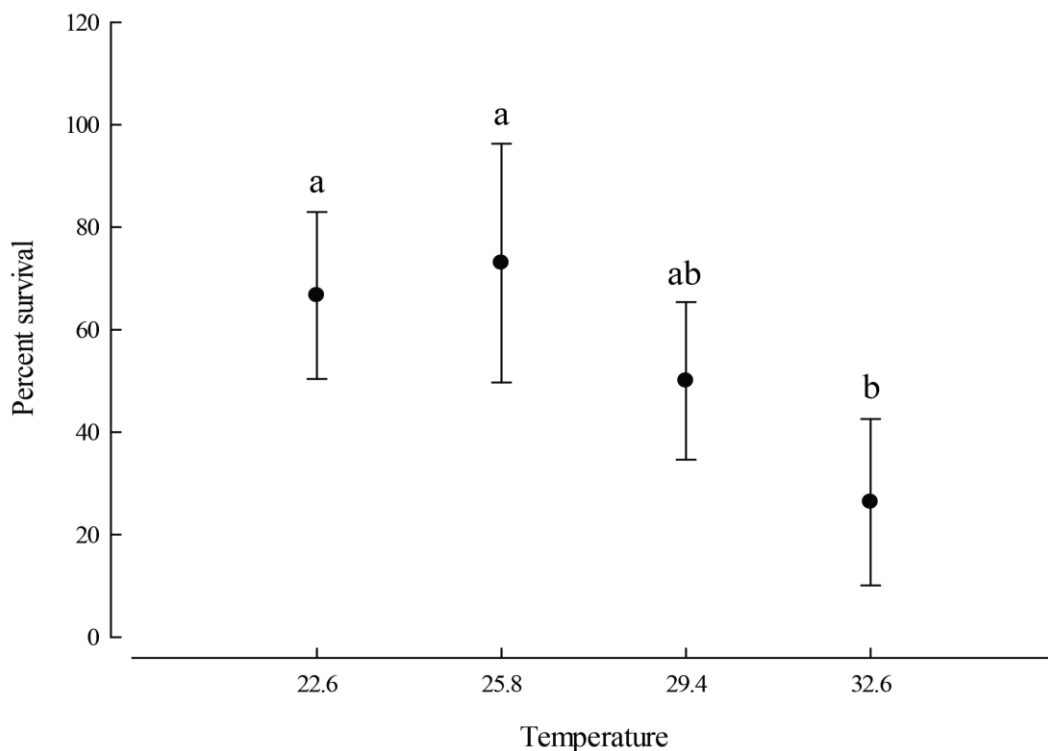


Figure 3.4: Mean percentage survival ($\pm 95\%$ CL) of *Metapenaeus dalli* larvae during to development from Nauplius VI to Mysis I at four different water temperatures. Letters above error bars denote groups of samples identified by Tukey's HSD ($p < 0.05$). Taken from Crisp *et al.* (2017).

Sediment composition

To test whether juvenile *M. dalli* prefer different sediment types, tank experiments were conducted, under controlled laboratory conditions, in which prawns were exposed to two different sediments types from the Swan-Canning

Estuary (Garratt Road Bridge and Dalkeith; Fig. 3.1). Chi-square tests demonstrated that *M. dalli* preferred the sediment composition of Dalkeith over that at Garratt Road Bridge (see Bennett, 2014 for full details). As it is hard to construct and test an *a priori* hypothesis for the response of post-larval *M. dalli* to POM and each of the Wentworth grain sizes, the data for each sediment composition variable were square-root transformed and subjected to Principal Component Analysis. This test was used to objectively determine sites with similar sediment composition and place them on a linear scale, *i.e.* a principal component, PC1, (Leonard *et al.*, 2006; Tweedley *et al.*, 2015). The PC1 scores for each site range from ~ -40 to ~ 140 (see later) with the scores for Daliketh (~ -25) and Garratt Road Bridge (~ 50) used to determine the orientation of the scores, *i.e.* that sites with negative scores were considered to have a good sediment composition for post-larval *M. dalli*. These PC1 scores were inversed, so that positive scores indicated a good release site, and standardised to produce a score of 0-100 for each site. This standardised value was used to represent sediment composition in the model.

Abundance of conspecifics and competitors

The abundances of (i) all individuals of *M. dalli*, (ii) solely gravid female *M. dalli* and (iii) all *P. latisulcatus* were converted to a density 500 m². Each of these variables was standardised (0-100) by dividing the density for a replicate by the maximum value recorded for the variable and multiplying by 100. While the densities of *M. dalli* were considered to be a positive metric (as densities had not reached the point where density-dependent effects are influential; see Broadley *et al.* 2017), densities of *P. latisulcatus* were considered a negative metric due to the density-dependent competition they impose. Thus, values for this variable were inversed by subtracting each score from 100, so that species with high abundances now had a score closer to 0 and *vice versa*, with these scores forming the basis of the competitor factor, while the scores for total *M. dalli* and gravid female *M. dalli* were used in the conspecific factor in the model.

Abundance of teleosts and scyphozoan predators

Each of the teleost and scyphozoan species considered likely to predate on post-larval *M. dalli* based on dietary analysis were assigned a 'predation score' ranging between 1, *i.e.* rarely likely to predate on post-larval *M. dalli* and, if so, only consume low numbers, and 10, *i.e.* likely to be a significant predator on post-larval *M. dalli* and able to consume larger numbers (Tables 3.2, 3.3). To reduce the number of variables in the model, only those teleost species with a predation score ≥ 3 were included, while the two scyphozoan predators were excluded as they would be less able to target the benthic post-larval than pelagic larval stages. Thus, the predatory species included in the model were the apogonid Western Gobbleguts (*Ostorhinchus rueppellii*), the atherinids Common Hardyhead (*Atherinomorus vaigiensis*), *A. elongata*, *C. mugiloides* and *L. presbyteroides* (noting that in the samples collected at night the last three species were unable to be distinguished and recorded as 'Atherindae'), the sparid Black Bream *Acanthopagrus butcheri* and the gobiid Yellowspotted Sandgoby (*Favonigobius punctatus*).

The replicate densities of each of these species (fish 100 m⁻²) in each site and month combination were averaged and multiplied by the predation scores of that species (Tables 3.2, 3.3) to calculate the Relative Predation Index (RPI), which aimed to determine the potential impact that species in that sample may have on the survival of hatchery-reared post-larval *M. dalli*. This quantitative index aimed to remove the bias of a species such as atherinids that may not, individually, predate on large amounts on post-larval *M. dalli*, but can occur in huge densities (*e.g.* Hoeksema *et al.* 2009) and likewise those species that may occur in lower numbers, such as *O. rueppellii*, but have been recorded consuming large number of post-larval *M. dalli* (*i.e.* the 300 prawns recorded in the stomach of one *O. rueppellii*; Poh, B., Murdoch University, unpublished data).

The RPI of each of the seven species was combined to give an overall teleost predator RPI before weighting took place. This method of weighting species was

based on their potential predation impact, rather than giving each an equal contribution to the model. Thus, it circumvented the problem of not all species being found in each sample, which as the absence of a predator results in a positive score, could produce an artificially high score.

Whereas maximum scores were used for the standardisation of the other variables in the model, it was believed that due to the highly schooling nature of fish, that standardisation using the 75th percentile value would be a more appropriate method. This would prevent standardising all scores to potentially an outlier in the data and was deemed preferable over a power transformation, *e.g.* square-root, fourth-root or $\text{Log}(x+1)$. The 75th percentile of the combined RPI was calculated and used in the standardisation process detailed above. Note that as this resulted in some values being > 100 these were modified to read 100, *i.e.* the maximum possible score. Finally, like the effect of competition by *P. latisulcatus*, predation was deemed to have a negative effect on the survival of post-larval *M. dalli* and thus the standardised combined RPI scores were inverted and used in the model to represent the predation factor.

3.2.4.2: Calculation of the SMART score

As mentioned above, this section focuses predominantly on the production and development of the SMART using the night data, as this is the most comprehensive data set. Differences in the day model and also a comparison of sites where both day and night sampling overlapped will be highlighted at the end of the section.

The standardised (and possibly inversed) data for each of the five factors, *i.e.* water quality (salinity and water temperature), sediment composition (PC1 score), competitors (density of *P. latisulcatus*), conspecifics (density of all *M. dalli* and gravid *M. dalli*) and predation (the combined RPI of the seven teleost species) were averaged to give a final SMART score which ranged from 0-100 (0 being totally unsuitable for the survival of hatchery-reared post-larval *M. dalli* and 100 being optimal) for each site and time. Note that in the case of water quality and

conspecifics where the factor is comprised of more than one variable, the average of the variables for a site x month combination was calculated and used as the value for the factor.

3.2.4.3: Analysis and interpretation of the SMART score

Although the study aimed to be able to distinguish among individual sites in a given month, there were not enough replicates (*i.e.* 1-2) at that level to enable a robust statistical interpretation of the results. This is because the development of SMART was not anticipated when the requisite sampling regimes were devised and, in any case, would have required the collection and processing of far larger numbers of samples, which may not have been financially viable. As a result, sites were pooled into regions based on their location in the estuary (see Fig. 3.1), which provided enough replicates for analysis at various levels.

Prior to undertaking statistical analysis, the SMART scores were assessed using the R software package (R Core Team, 2015) to ascertain the type of transformation required, if any, to meet the test assumptions. The extent of the linear relationship between \log_e (mean) and \log_e (standard deviation) of all groups of replicate samples was determined and then using slope criteria provided by Clarke and Warwick (2001) an appropriate level of transformation was selected. This analysis indicated that, in all cases, the values for the SMART scores did not require transformation.

Each of the following statistical analyses was performed using PRIMER v7 multivariate software package (Clarke and Gorley, 2015), with the PERMANOVA+ add on module (Anderson *et al.*, 2008). Although region was the main factor of interest, differences among months (November to March) and summers (2013/14, 2014/15 and 2015/16) were accounted for so that their confounding influence could be quantified.

The final SMART scores for the night sites were used to make a Euclidean distance matrix, which was, in turn, subjected to a three-way Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson *et al.*, 2008) to test

whether the SMART scores differed significantly among Region (7 levels; Entrance Channel-Upper Canning), Month (6 levels; November-March) and Year (3 levels; 2013/14, 2014/15 and 2015/16). The null hypothesis of no significant differences among each term was rejected if the significance level (p) was < 0.05 and the relative influence of each factor in the model was quantified using the magnitude of the mean squares. In the event that a significant difference was detected in a main effect or interaction term, a pairwise PERMANOVA was conducted to elucidate the levels of the term on the model that were responsible for the differences. The extent of any significant differences among *a priori* groups were determined by the magnitude of the test statistic (t).

Bar and line graphs were produced to provide a visual representation of the change in SMART scores among significant factors and/or interactions. Shade plots (Clarke *et al.*, 2014) were produced using PRIMER v7 to visually display the SMART scores, in combination with the values for each factor and its component variables to illustrate why samples received a good or bad SMART score. These plots are a simple visualisation of the frequency matrix, where a white space for a score/factor/variable demonstrates that the score/factor/variable had a score of 0 and thus the site/region/month/year was totally unsuitable for the release of hatchery-reared post-larval *M. dalli* and the depth of shading from grey to black is linearly proportional to the score for that factor/variable. Black cells indicate that the score was 100 and thus that site/region/month/year was the optimal place or time to release the cultured prawns. Note that although the PERMANOVA tests were conducted at the region level, the main interest in the SMART score is at the site level and thus some of the shade plots (which are a data visualisation tools and not a statistical test) show data at this finer spatial scale, albeit from fewer replicates. Note also that the values for all factors and variables are those used in the model except for the individual components of the predation factors, these have been standardised to place them on a common scale with the other variables (*i.e.* 0-100).

During the day, the SMART score was calculated using only the water quality, sediment composition and predation factors, as abundance data for penaeids, which are used in the competitor and conspecific factors, were not recorded. As data for the variables in these factors was only collected in one month that overlapped with the night sites and in a single replicate, there were only three scores per site over the three years. To enable statistical analysis, the data for the regions were pooled across the three years to be able to test for differences in SMART score among regions.

Finally, a comparison between the SMART scores for those eight sites where data was collected both during the night and day was undertaken. The aim of this was to indicate whether day or night releases would facilitate better survival of post-larval *M. dalli*. As the focus of this analysis was to determine differences in optimal release site during day and night, rather than spatially across the estuary only the predation factor was included. This decision was made because, as mentioned above, the abundance of penaeids was not recorded during the day. Moreover, sediment composition was considered unlikely to undergo a diel change and, although water quality would change during a 24 hour cycle, this factor was to be more effective for describing spatial variation across the estuary rather than fine scale diel differences, especially as *M. dalli* released during the day would be exposed to the night-time temperatures in a few hours and *vice versa*.

3.3. Results

The results have been written in two major sections. The first summarises the results for each of the variables included in the model to establish the context for understanding the results of the SMART and the second refers to the results of the SMART.

3.3.1 Variation in individual factors

3.3.1.1. Night

Water quality

Water temperature followed a similar monthly pattern during each of the three years rising to a peak in January and February and typically declining in March (Fig. 3.5). In the summer of 2015/16, the temperatures were slightly warmer than those in the preceding year, while those regions located in Melville Water and the Entrance Channel were usually 2°C cooler than those further upstream (*i.e.* 21-25; Fig. 3.5a vs 23-27°C; Fig. 3.5b).

Salinity increased progressively from November to March in most regions during each of the three years, except in Perth Water between the November (~25) and December (~9) of 2013, when salinity declined markedly, before returning to ~34 in January (Fig. 3.6). A similar, albeit, less pronounced trend occurred in the Entrance Channel in 2015/16. Typically, salinity remained fairly consistent in this region and the two in Melville Water ranging 28 to 37, whereas in the other regions it ranged from 15 in November to 37 in March (Fig. 3.6). In almost all months, salinity was lowest in the Middle Swan Estuary and sometimes markedly so.

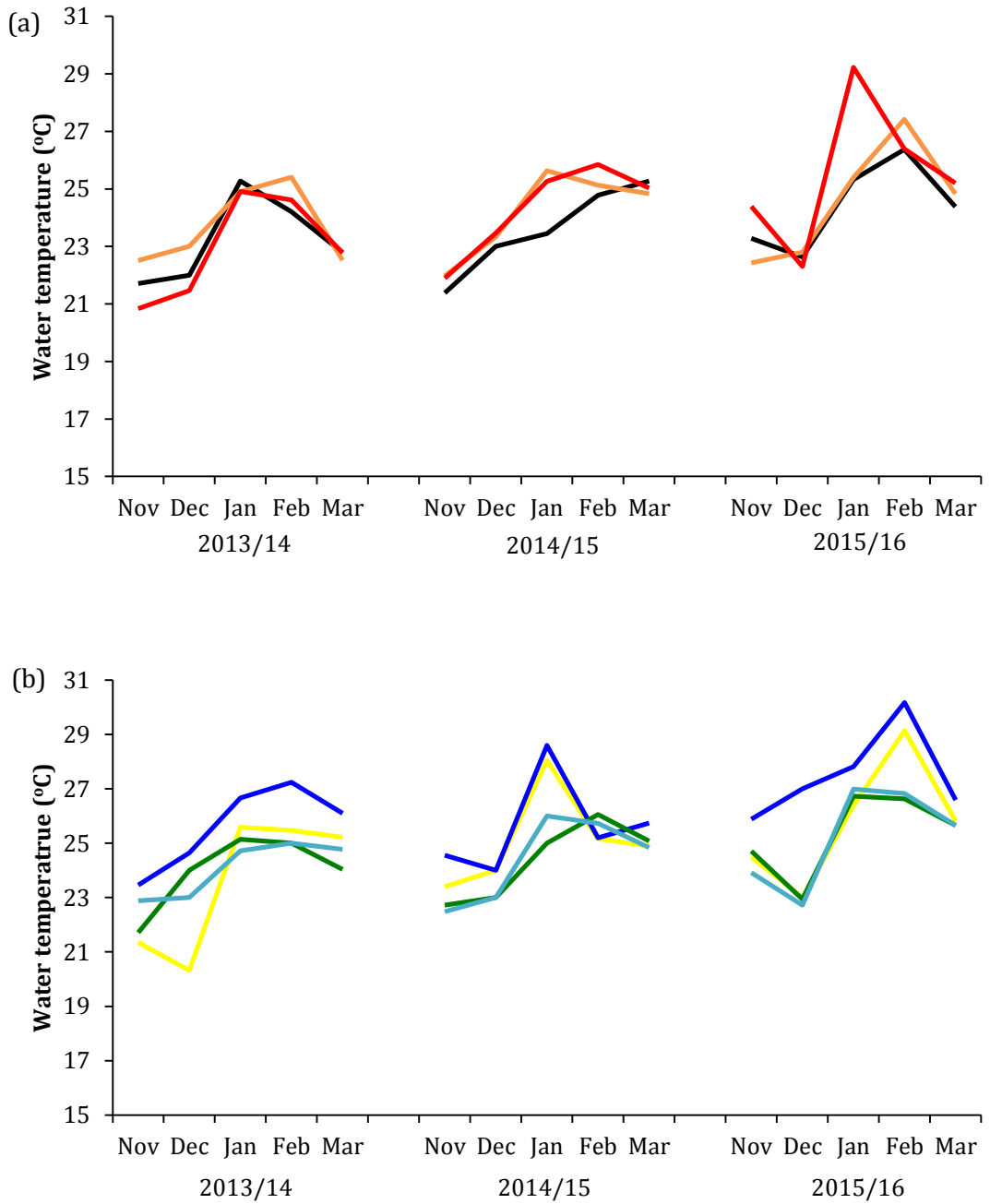


Figure 3.5: Mean water temperature (°C) recorded at night in the nearshore waters of each of the seven regions of Swan-Canning Estuary between November and March in three consecutive years. Regions; Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●).

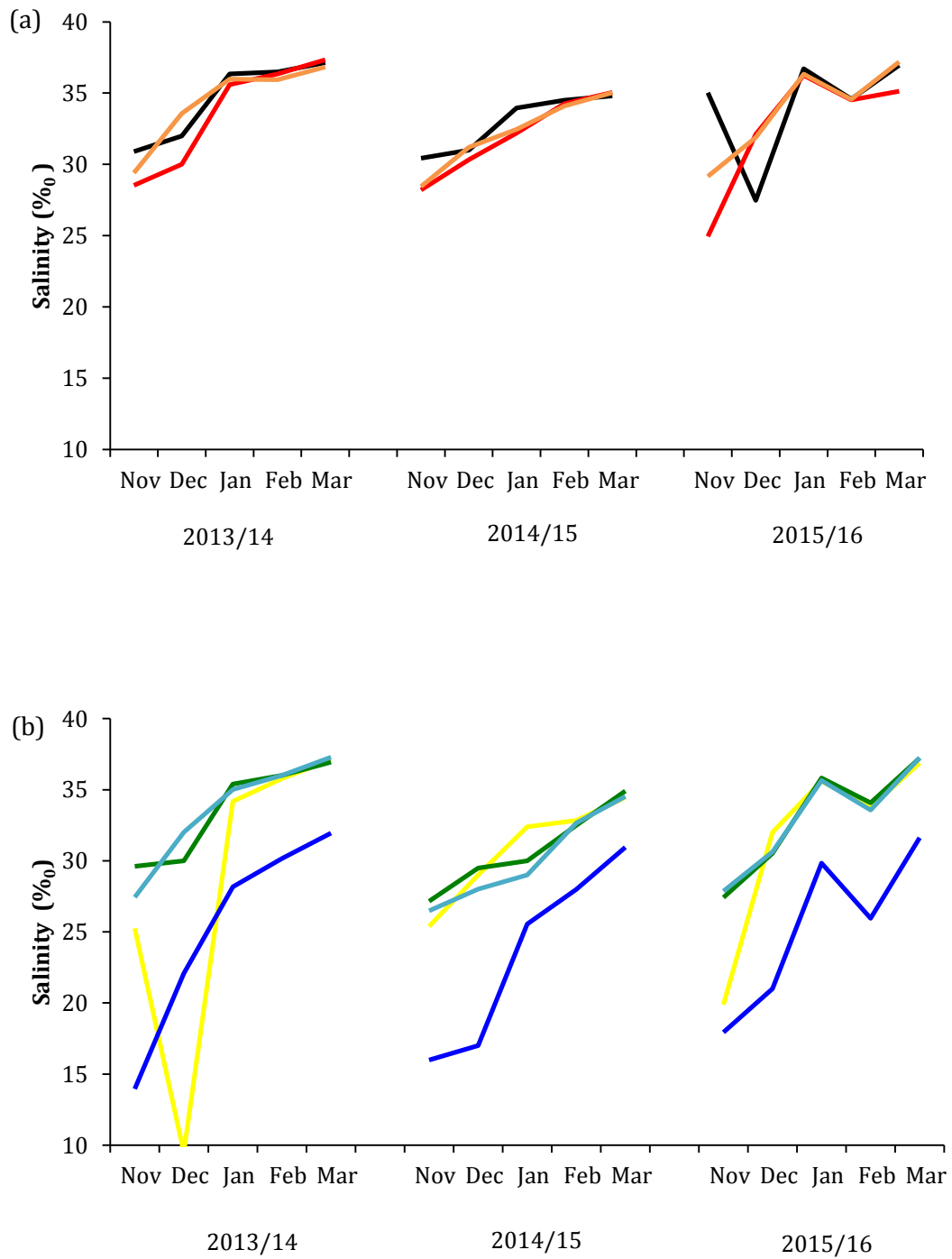


Figure 3.6: Mean salinity (‰) recorded at night in the nearshore waters of each of the seven regions of Swan-Canning Estuary between November and March in three consecutive years. Regions; Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●).

Sediment composition

Principal Component Analysis of the sediment composition data for the 16 nearshore sites in the Swan-Canning Estuary demonstrated that 55% of the variation was explained by PC1. Those sites in the Entrance Channel had the greater PC1 scores (90-125), which was due to this region exhibiting the largest percentage of particulate organic material (POM) and proportion of the 125, 63 and <63 μm inorganic grain sizes (Fig. 3.7, 3.8). Similarly, the sites with the next greatest PC1 values were those in the Middle Swan Estuary, due to large percentage contributions of POM. The remaining regions, had a more similar sediment composition, being dominated by the 500 and 250 μm grain sizes, with those sites in Perth Water typically containing large contributions of the former grain size. The PC1 axis provided good separation of the Garratt Road Bridge (39) and Dalkeith (-34) sites, which were the two sediments types that Bennett (2014) used for sediment preference experiments. The sediment at Garratt Road Bridge was characterised by a large amount of POM, while that at Dalkeith, comprised greater proportions of the 500 μm grain size (Fig. 3.8).

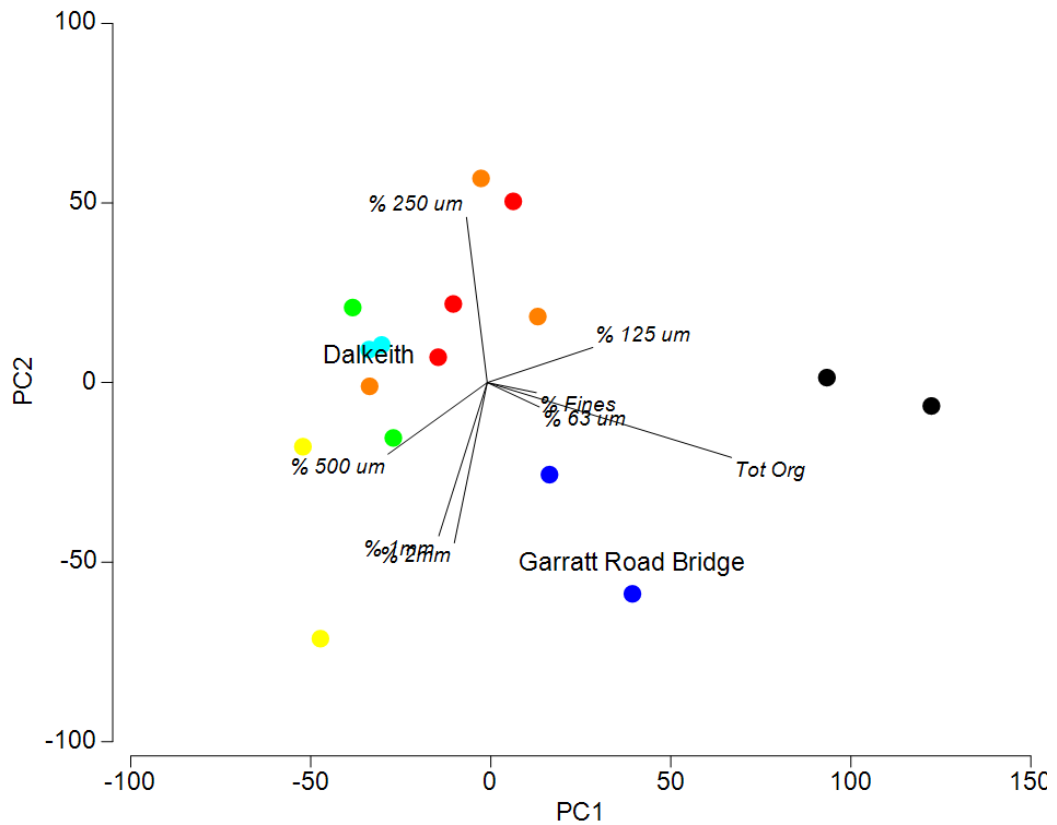


Figure 3.7: Principal Component Analysis plot of the mean sediment composition at each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in February 2014. Vectors have been overlaid showing trends in the percentage contribution of particulate organic matter (Tot Org) and each of the inorganic grain sizes (*i.e.* 2 mm, 1 mm, 500 μ m, 250 μ m, 125 μ m and 63 μ m and < 63 μ m [fines]). Sites coded for region, *i.e.* Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●). Data for the analysis taken from Bennett (2014).

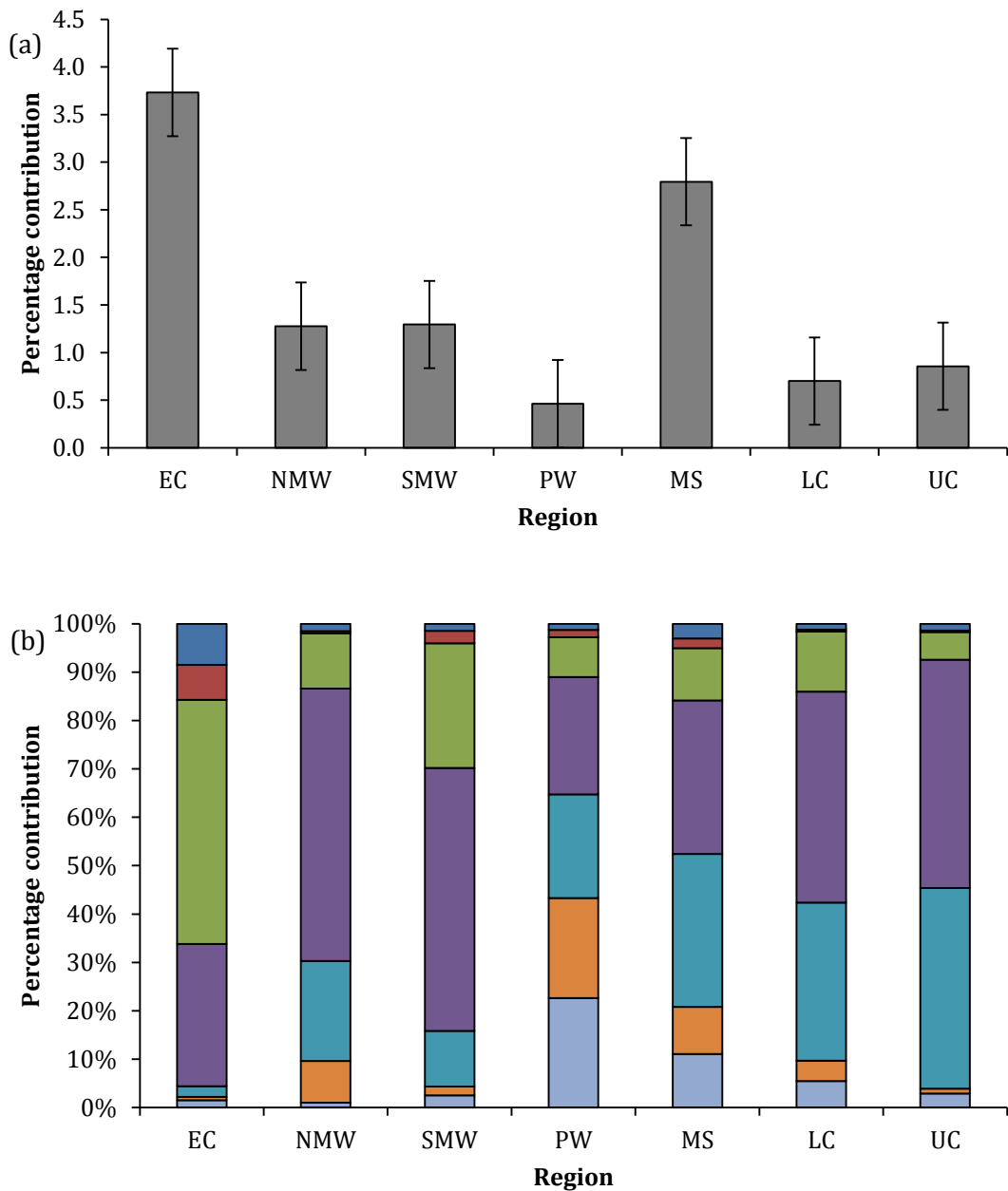


Figure 3.8: Mean percentage contribution of (a) particulate organic matter (b) and various inorganic grain sizes to the sediment at each of the seven regions in the nearshore waters of the Swan-Canning Estuary. Inorganic grain sizes; 2 mm (□), 1 mm (□), 500 μm (□), 250 μm (□), 125 μm (□), 63 μm (□) and < 63 μm [fines] (□). Regions; Entrance Channel (EC), South Melville Water (SMW), North Melville Water (NMW), Perth Water (PW), Lower Canning Estuary (LC), Upper Canning Estuary (UC) and the Middle Swan Estuary (MS). Data for the analysis taken from Bennett (2014).

Densities of penaeids

The Western King Prawn *Penaeus latisculatus* was found predominantly in the Entrance Channel and North and South shores of Melville Water, with its

densities greatest in the first region (up to 12 individuals 500 m⁻²; Fig. 3.9). This species was only infrequently recorded and, if so, in low densities in Perth Water and the Lower Canning Estuary (maximum density of 2 individuals 500 m⁻²), thus its density and frequency of occurrence declined with increased distance upstream. No clear patterns were evident in either among month in each year or between years (Fig. 3.9).

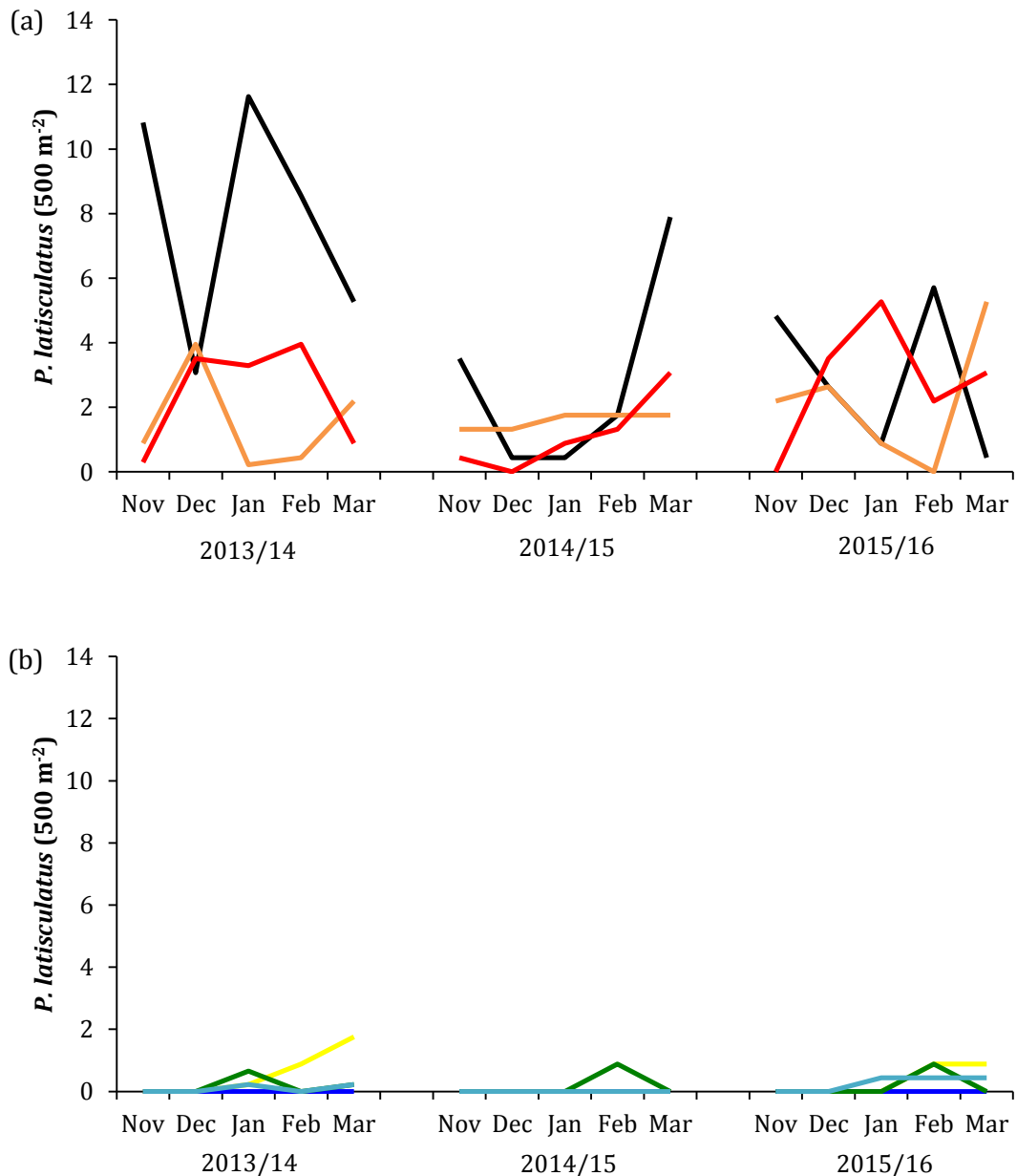


Figure 3.9: Density of the Western King Prawn *Penaeus latisulcatus* (individuals 500 m⁻²) to the sediment at each of the seven regions in the nearshore waters of the Swan-Canning Estuary monthly between November and March of 2013/14, 2014/15 and 2015/16. Regions; Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●).

The densities of *M. dalli* showed a different spatial pattern to those of *P. latisulcatus*, with the regions further upstream of Melville Water, such as the Lower and Upper Canning Estuary and Perth Water harbouring the greatest densities of *M. dalli* (Fig. 3.10). Densities fluctuated within each year, typically exhibiting two peaks, with the first in November/December and the second in February. Densities of gravid *M. dalli* exhibited a similar pattern to the total population, with the exception that relatively larger numbers were found at sites in North Melville Water, and particularly so during 2015/16 (Fig. 3.11). While two peaks in the densities of gravid *M. dalli* were also recorded in each year, in some cases, *e.g.* Lower Canning Estuary in 2013/14 and North Melville Water in 2015/16, the first peak (*i.e.* ~8 and ~7 individuals 500 m⁻², respectively) was considerably larger than the second (*i.e.* ~2 and ~1 individuals 500 m⁻², respectively; Fig. 3.11).

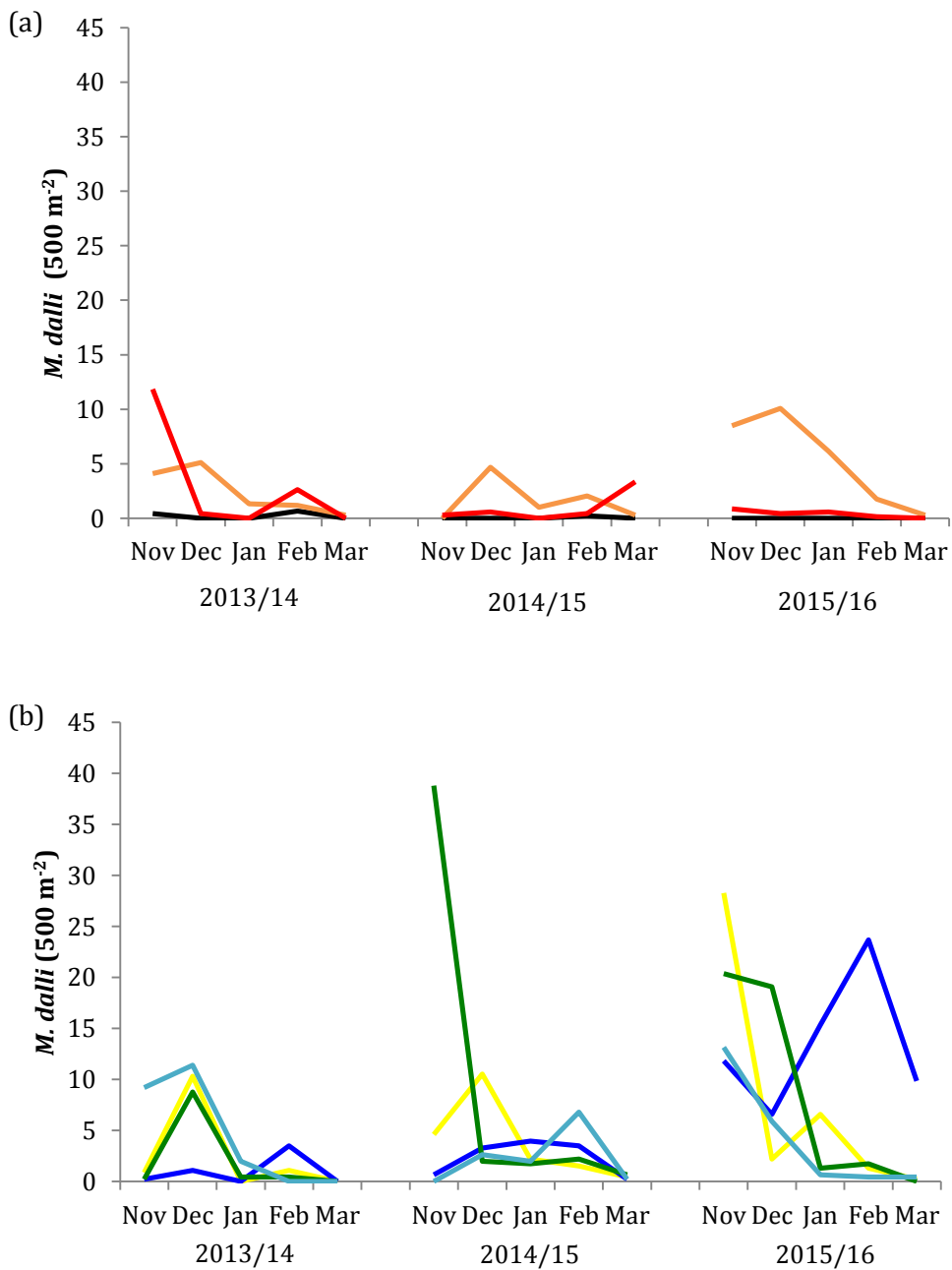


Figure 3.10: Density of the Western School Prawn *Metapenaeus dalli* (individuals 500 m⁻²) at each of the seven regions in the nearshore waters of the Swan-Canning Estuary monthly between November and March of 2013/14, 2014/15 and 2015/16. Regions; Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●).

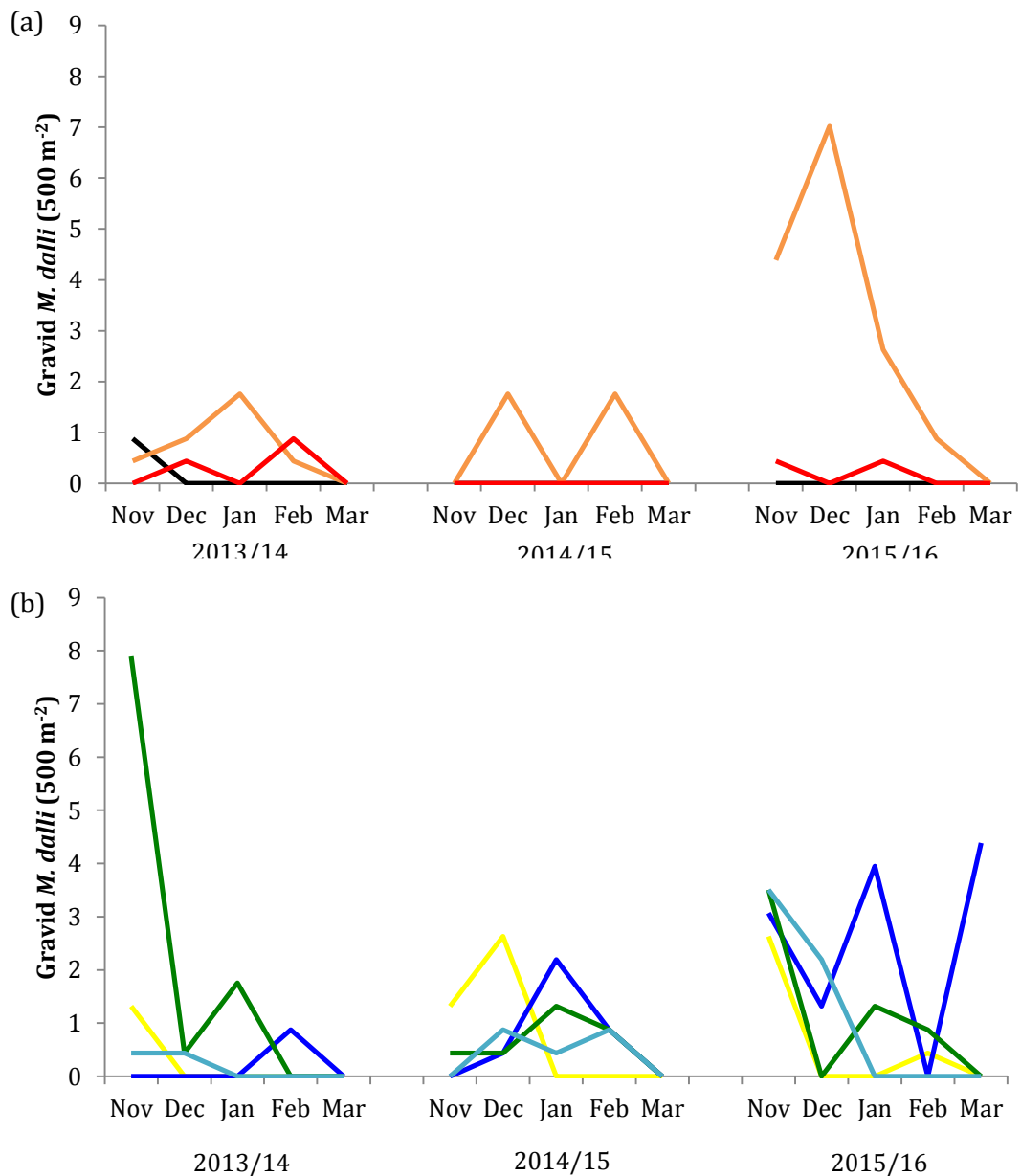


Figure 3.11: Density of the gravid Western School Prawn *Metapenaeus dalli* (individuals 500 m⁻²) at each of the seven regions in the nearshore waters of the Swan-Canning Estuary monthly between November and March of 2013/14, 2014/15 and 2015/16. Regions; Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●).

Densities of teleost and scyphozoan predators

Nineteen of the 41 fish species recorded during night-time sampling in the nearshore waters of the Swan-Canning Estuary were identified as predators, or considered likely to be predators of post-larval *M. dalli*, based on stomach content analysis (Table 3.2; Fig. 3.13). The Atherinidae (grouped *A. elongata*, *C. mugiloides*

and *L. presbyteroides*) were the most abundant species, comprising ~89% of all fish recorded. Other abundant fish included the Banded Toadfish (*Torquigener pleurogramma*) and the Western Gobbleguts (*Ostorinchus rueppellii*), which represented 5 and 3% of the total number of fish.

The Brown Jellyfish (*Phyllorhiza punctata*) represented 80% of the total abundance of scyphozoans (~12 individuals 100 m⁻²), with the Moon Jellyfish (*Aurelia aurata*) comprising the remaining 20% (~4 individuals 100 m⁻²; Table 3.2).

The scoring of predators for the model are based on the dietary studies of Poh (Murdoch University, unpublished data), who examined the gut contents of numerous teleost species following the release of post-larval *M. dalli* at Matilda Bay. *Ostorinchus rueppellii* was the predator that contained the largest number of prawns (up to 300 post-larval *M. dalli* in a single fish) and fed on them most consistently and thus was identified as the most significant threat to released *M. dalli* and assigned a predation score of 10 (Table 3.2). This species was consistently present in high densities within each month for all regions, except the Entrance Channel (Fig. 3.12).

The second highest predation score (6) was assigned to the Atherinid (*Atherinomorus vaigiensis*). Whilst consistent in its predation of *M. dalli* during the study period, *A. vaigiensis* was not observed to have predated as heavily on the released prawns as *O. rueppellii*. This species was not particularly abundant for long periods throughout any region apart from in the Lower Canning Estuary, where it was present in each month of each year (Fig. 3.12).

The Yellowspotted Sandgoby *Favonigobius punctatus* was assigned a predation score of 3 as it was found to predate on *M. dalli* in small amounts, as well as other small crustaceans. Likewise, *Acanthopagrus butcheri* was assigned a score of 3, observed to have directly predated on *M. dalli*, albeit only fish of this species <100 mm in total length were found with *M. dalli* in their stomachs and only in

small amounts. Both of these species were most abundant in the Perth Water and the Middle Swan Estuary (Fig. 3.12).

The final group to be assigned a predation score > 3 were the Atherinidae. While predating predominantly on *M. dalli* and other small crustaceans, a high percentage of stomach contents from this family were empty, therefore not contributing to the percentage composition of stomach contents. Further, when *M. dalli* was recorded in stomach contents of members of this group, they occurred in small numbers (*i.e.* 1 or 2). As a result, although their densities were very high, they were assessed as unlikely to have a large impact on survival of released *M. dalli*. Like *O. rueppellii*, the atherinids were present throughout the estuary in all sampling periods, except in the Middle Swan Estuary where they were not found in high abundance in any month (Fig. 3.12). All other species were assigned a predation score of ≤ 2 based on the fact that their diet only included a small percentage of *M. dalli* and/or other small crustaceans.

Table 3.2: Density 100 m⁻² (D) and percentage contribution (%) to total density of all teleost and scyphozoan species deemed to predate on released *M. dalli* or have the potential to predate on them. A predation score (P) ranging between 1 (low) and 10 (very high) is assigned to each species based on the risk that species presents for predation on released post-larval *M. dalli*. Species with a predation score ≥ 3 are shaded in grey. Atherinidae is a combination of *A. elongata*, *C. mugiloides* and *L. presbyteroides* as these species require laboratory classification.

Teleost species	Common name	P	Total		2013/14		2014/15		2015/16	
			D	%	D	%	D	%	D	%
Atherinidae	Hardyheads	3	936.5	89.8	395	89.9	170.8	87.2	370.6	90.5
<i>Torquigener pleurogramma</i>	Banded Toadfish	1	50.6	4.9	25.3	5.8	10.2	5.2	15.1	3.7
<i>Ostorhinchus rueppellii</i>	Western Gobbleguts	10	33.2	3.2	7.1	1.6	8.1	4.1	18	4.4
<i>Spratelloides robustus</i>	School Whiting	2	4.5	0.4			3.6	1.8	0.9	0.2
<i>Favonigobius punctatus</i>	Sandgoby	3	3.7	0.4	2.5	0.6	0.5	0.3	0.7	0.2
<i>Atherinomorus vaigiensis</i>	Common Hardyhead	6	3.3	0.3	1.9	0.4	0.7	0.4	0.7	0.2
<i>Pelates octolineatus</i>	Striped Grunter	1	2.9	0.3	2.6	0.6	0.1	0.1	0.2	>0.1
<i>Favonigobius lateralis</i>	Southern Longfin Goby	1	2.6	0.2	2.5	0.6	0.1	0.1	0.1	>0.1
<i>Acanthopagrus butcheri</i>	Black Bream	3	1.8	0.2	1	0.2	0.2	0.1	0.6	0.1
<i>Pseudogobius olorum</i>	Swan River Goby	2	1.4	0.1	0.2	0	0.5	0.3	0.7	0.2
<i>Amniataba caudavittata</i>	Yellowtail Grunter	1	0.7	0.1	0.3	0.1	0.2	0.1	0.2	>0.1
<i>Mugil cephalus</i>	Sea Mullet	1	0.6	>0.1	0.1	>0.1	0.7	0.4	1.3	0.3
<i>Engraulis australis</i>	Australian Anchovy	1	0.5	>0.1	0.3	0.1	0.2	0.1	0.1	>0.1
<i>Rhabdosargus sarba</i>	Tarwhine	2	0.3	>0.1	0.3	0.1				
<i>Hyporhamphus melanochir</i>	Southern Sea Garfish	1	0.2	>0.1	0.2	>0.1				
<i>Haletta semifasciata</i>	Blue Weed Whiting	1	0.2	>0.1	0.2	>0.1				
<i>Hyperlophus vittatus</i>	Sandy Sprat	2	0.1	>0.1					0.1	>0.1
<i>Gerres subfasciatus</i>	Common Silver Belly	1	0.1	>0.1					0.1	>0.1
<i>Silago maculata</i>	Trumpeter Whiting	1	0.1	>0.1					0.1	>0.1
Number of Species			19		15		13		15	
Total density			1,043		440		196		410	
Total no. fish			232,806		122,310		35,712		74,784	

Scyphozoan Species		D	%	D	%	D	%	D	%
<i>Phyllorhiza punctata</i>	Brown Jellyfish	11.6	80	0.6	40	1.4	80	9.6	80
<i>Aurelia aurata</i>	Moon Jellyfish	3.8	20	0.8	60	0.3	20	2.8	20
Total density		15.4		1.4		1.7		12.4	
Total no. scyphozoans		2,929		371		305		2,253	

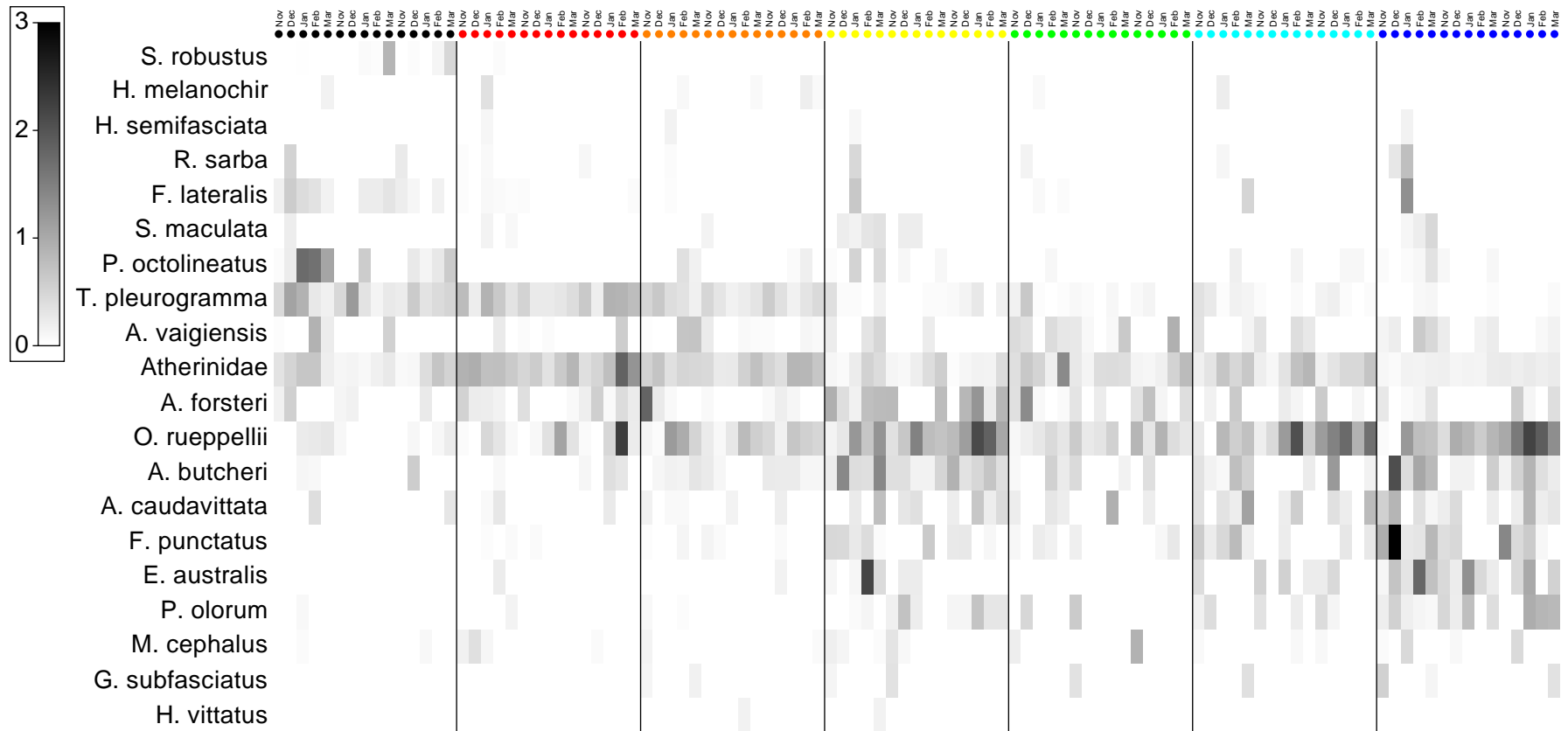


Figure 3.12: Shade plot dispersion weighted and square-root transformed densities of each of the 19 species identified to predate or potentially predate on *M. dalli* in each of the seven regions during each month (November-March) of each year (2013/14, 2014/15 and 2015/16) in the Swan-Canning Estuary. White space denotes the absence of a species, with the grey scale representing the pretreated abundances. Regions; Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●).

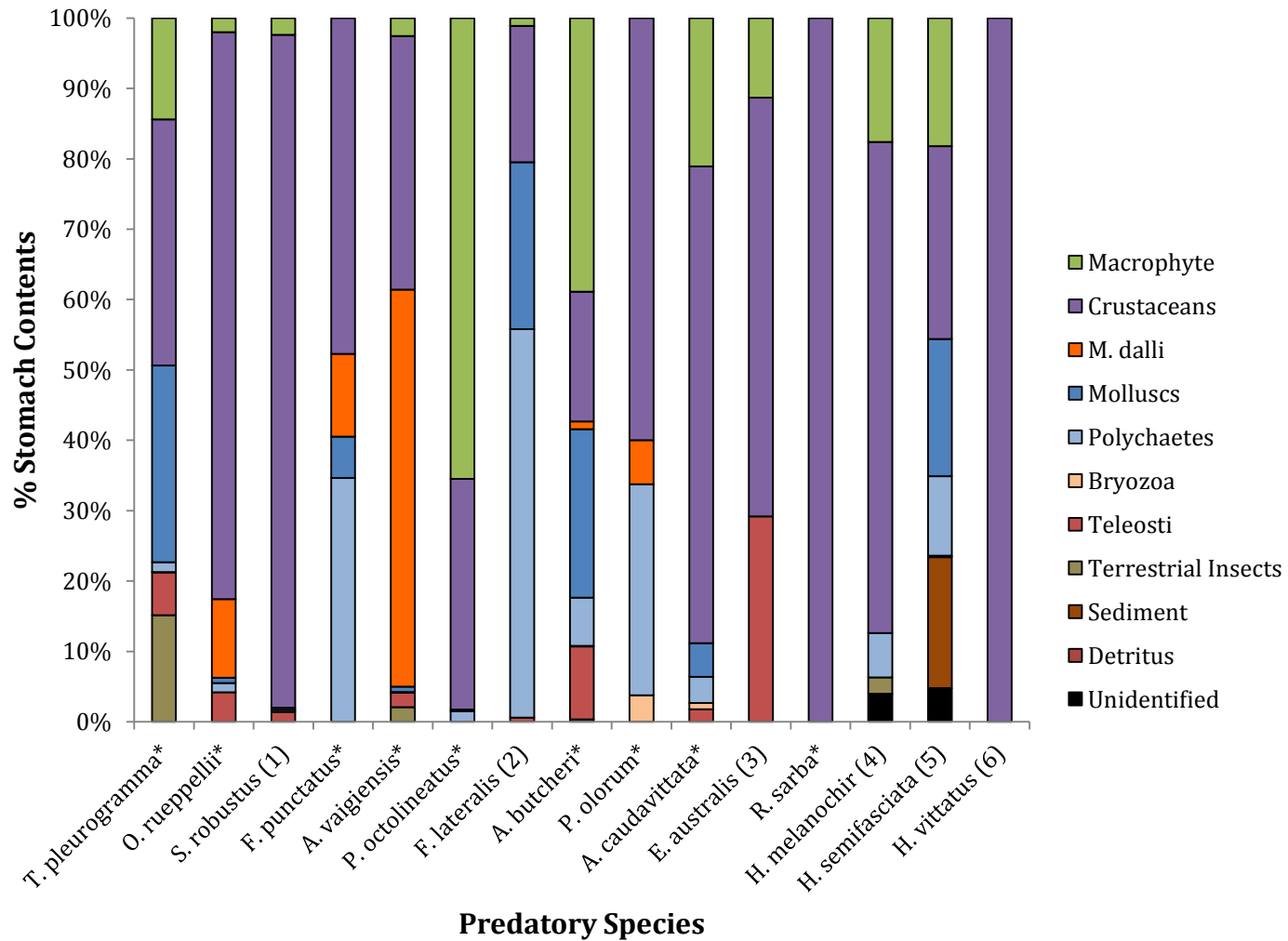


Figure 3.13: Percentage contribution of dietary items to the stomachs of the 19 teleost species identified to predate or potentially predate upon *M. dalli*. * indicates those species that were sampled by Poh (Murdoch University, unpublished data) after the release of *M. dalli* into the area, separating *M. dalli* from other crustaceans in the stomach contents. The numbers represent studies by others that were used to identify the stomach contents of the fish not examined by Poh. (¹ Dube and Kamusoko, 2013, ² Humphries and Potter, 1993, ³ Rao and Babu, 2013, ⁴ MacArthur and Hyndes, 2007, ⁵ Robertson and Klumpp, 1983, ⁶ MacArthur and Hyndes, 2007).

3.3.1.2: Day Results

Water quality

Spatial patterns in water temperature patterns were similar to those recorded during the night, with higher temperatures recorded in Perth Water and the Middle Swan Estuary (Fig. 3.14). Water temperatures in most regions were slightly cooler in 2015/16 than in the previous two years. Comparisons of water temperatures in each region during the day were typically ~2-3 °C higher than in the same region at night in 2013/14 and 2014/15, although they were similar in 2015/16 (*cf* Figs 3.5, 3.14).

Salinities were greatest in the regions located in Melville Water, *i.e.* 33-36, compared to 28-34 in the more upstream regions (Fig. 3.15). There were no observable differences in salinities in January in each year between the day and night (*cf.* Figs 3.6, 3.15).

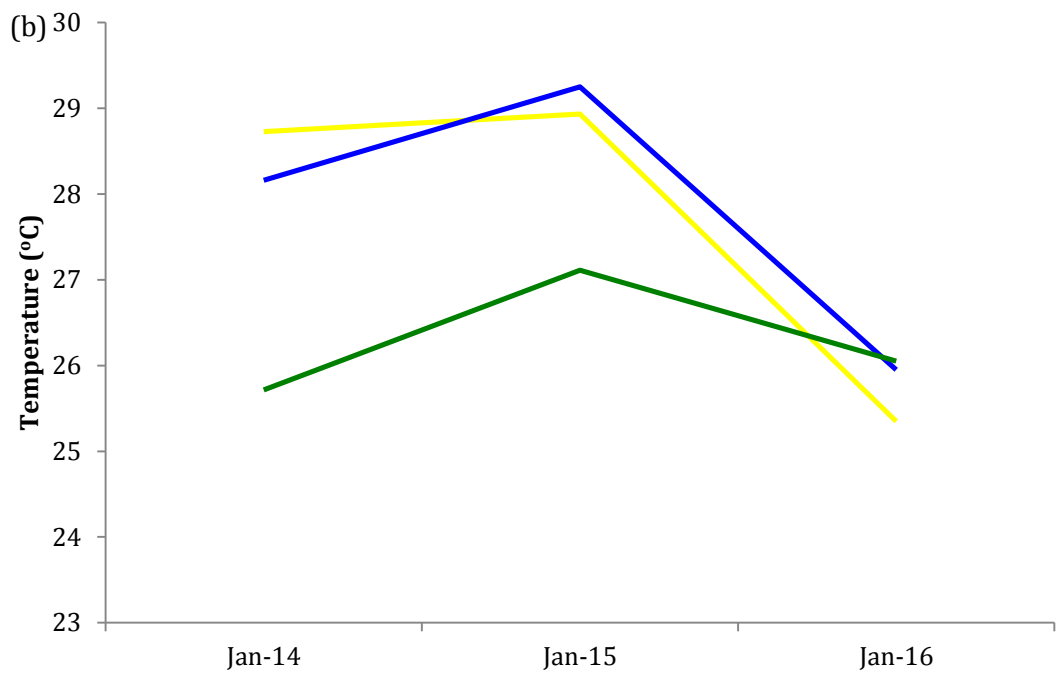
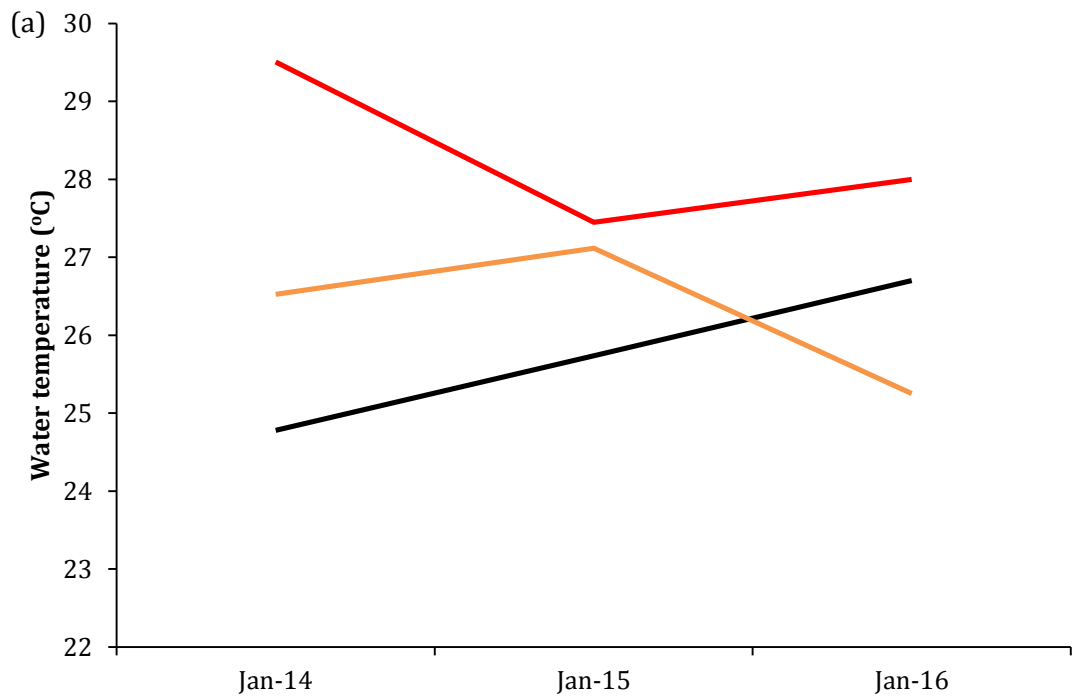


Figure 3.14: Mean water temperature (°C) recorded at day in the nearshore waters of each of the six regions of Swan-Canning Estuary during January in three consecutive years. Regions; Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●) and the Middle Swan Estuary (●).

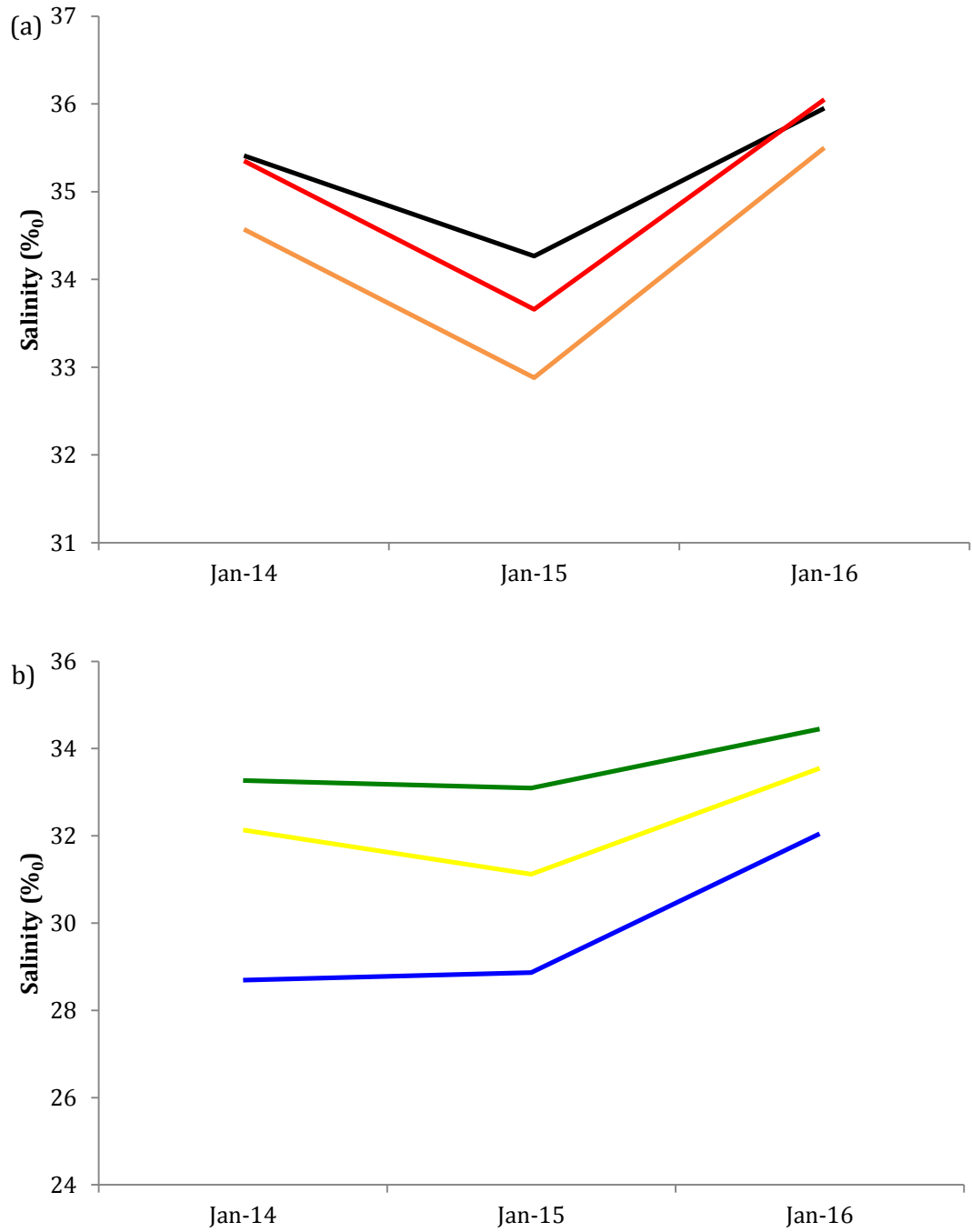


Figure 3.15: Mean salinity recorded at day in the nearshore waters of each of the six regions of Swan-Canning Estuary during January in three consecutive years. Regions; Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●) and the Middle Swan Estuary (●).

Sediment composition and penaeid abundance

Sediment composition data from Bennett (2014) were used for both the night and day (see sections 3.2. and 3.3.1.1). Penaeid abundance was not recorded during the day as the species of interest in the Swan-Canning Estuary are buried during the day and thus not vulnerable to capture in nets.

Density of teleost predators

Twenty-eight teleost species recorded during the day were identified as potential predators of post-larval *M. dalli* (Table 3.3; Fig. 3.17). The predation scores assigned during the night were the same as those assigned to each species during the day. Similar to the night, the atherinids group comprising of *A. elongata*, *C. mugiloides* and *L. presbyteroides* were abundant (329, 299 and 78 individuals 100 m⁻², respectively) and together contributed 49% to the total fish density (Table 3.3). *Ostorhinchus rueppellii* was substantially lower densities during the day than the night and was also more patchily distributed throughout the estuary, with *Favinogobius punctatus* exhibiting the reverse trends (Fig. 3.16).

Table 3.3: Density 100 m⁻² (D) and percentage contribution (%) to total density of all teleost species deemed to predate on released *M. dalli* or have the potential to predate on them. A predation score (P) ranging between 1 (low) and 10 (very high) is assigned to each species based on the risk that species presents for predation on released post-larval *M. dalli*. Species with a predation score ≥ 3 are shaded in grey. Atherinidae is a combination of *A. elongata*, *C. mugiloides* and *L. presbyteroides* as these species require laboratory classification.

Species	Common Name	P	Total		Summer 2014		Summer 2015		Summer 2016	
			D	%	D	%	D	%	D	%
<i>Atherinosoma elongata</i>	Elongate Hardyhead	3	328.5	23%	157.1	32%	110.5	21%	60.3	14%
<i>Craterocephalus mugiloides</i>	Spotted Hardyhead	3	298.9	21%	75.4	16%	101	19%	122.2	28%
<i>Leptatherina wallacei</i>	Western Hardyhead	2	278.8	19%	38.1	8%	190.8	36%	49.4	11%
<i>Favonigobius punctatus</i>	Sandgoby	3	100.8	7%	76.8	16%	10	2%	13.8	3%
<i>Leptatherina presbyteroides</i>	Swan River Hardyhead	3	77.5	5%	0	0%	3.7	1%	73.9	17%
<i>Acanthopagrus butcheri</i>	Black Bream	3	76	5%	35.1	7%	10.8	2%	30	7%
<i>Pseudogobius olorum</i>	Swan River Goby	2	61.3	4%	46.5	10%	11.4	2%	3.3	1%
<i>Engraulis australis</i>	Australian Anchovy	2	44.5	3%	2.2	0%	22.8	4%	19.4	5%
<i>Amniataba caudavittata</i>	Yellowtail Grunter	2	43	3%	22.5	5%	8	2%	12.5	3%
<i>Torquigener pleurogramma</i>	Banded Toadfish	1	42.6	3%	16	3%	17	3%	9.6	2%
<i>Gambusia holbrooki</i>	Eastern Gambusia	2	31.2	2%	1.2	0%	25.9	5%	4.1	1%
<i>Pelates octolineatus</i>	Striped Grunter	1	24.1	2%	1.3	0%	3.6	1%	19.1	4%
<i>Ostorhinchus rueppellii</i>	Western Gobbleguts	10	19.2	1%	10.7	2%	2.9	1%	5.5	1%
<i>Atherinomorus vaigiensis</i>	Common Hardyhead	6	12.3	1%	0.8	0%	5.8	1%	5.8	1%
<i>Afurcagobius suppositus</i>	Southwest Goby	1	1.6	0%	1.1	0%	0	0%	0.5	0%
<i>Sillago burrus</i>	Trumpeter Whiting	1	1.4	0%	0	0%	0.6	0%	0.8	0%
<i>Haletta semifasciata</i>	Blue Weed Whiting	1	0.3	0%	0	0%	0	0%	0.3	0%
<i>Rhabdosargus sarba</i>	Tarwhine	2	0.3	0%	0	0%	0.1	0%	0.2	0%
<i>Urocampus carinirostris</i>	Hairy Pipefish	1	0.3	0%	0	0%	0	0%	0.3	0%
<i>Elops machnata</i>	Tenpounder	1	0.2	0%	0.2	0%	0	0%	0	0%
<i>Hydrocynus vittatus</i>	Sandy Sprat	2	0.2	0%	0.2	0%	0	0%	0	0%
<i>Sillago schomburgkii</i>	Yellowfin Whiting	1	0.2	0%	0.1	0%	0	0%	0.1	0%
<i>Pseudorhombus jenynsii</i>	Short Toothed Flounder	1	0.1	0%	0.1	0%	0	0%	0	0%
<i>Stigmatopora nigra</i>	Widebody Pipefish	1	0.1	0%	0	0%	0	0%	0.1	0%
Number of Species			24		31		21		30	
Totals	Average Total Density		1443.3		485.3		524.9		431.2	
	Total no. Fish		1688		569		614		505	

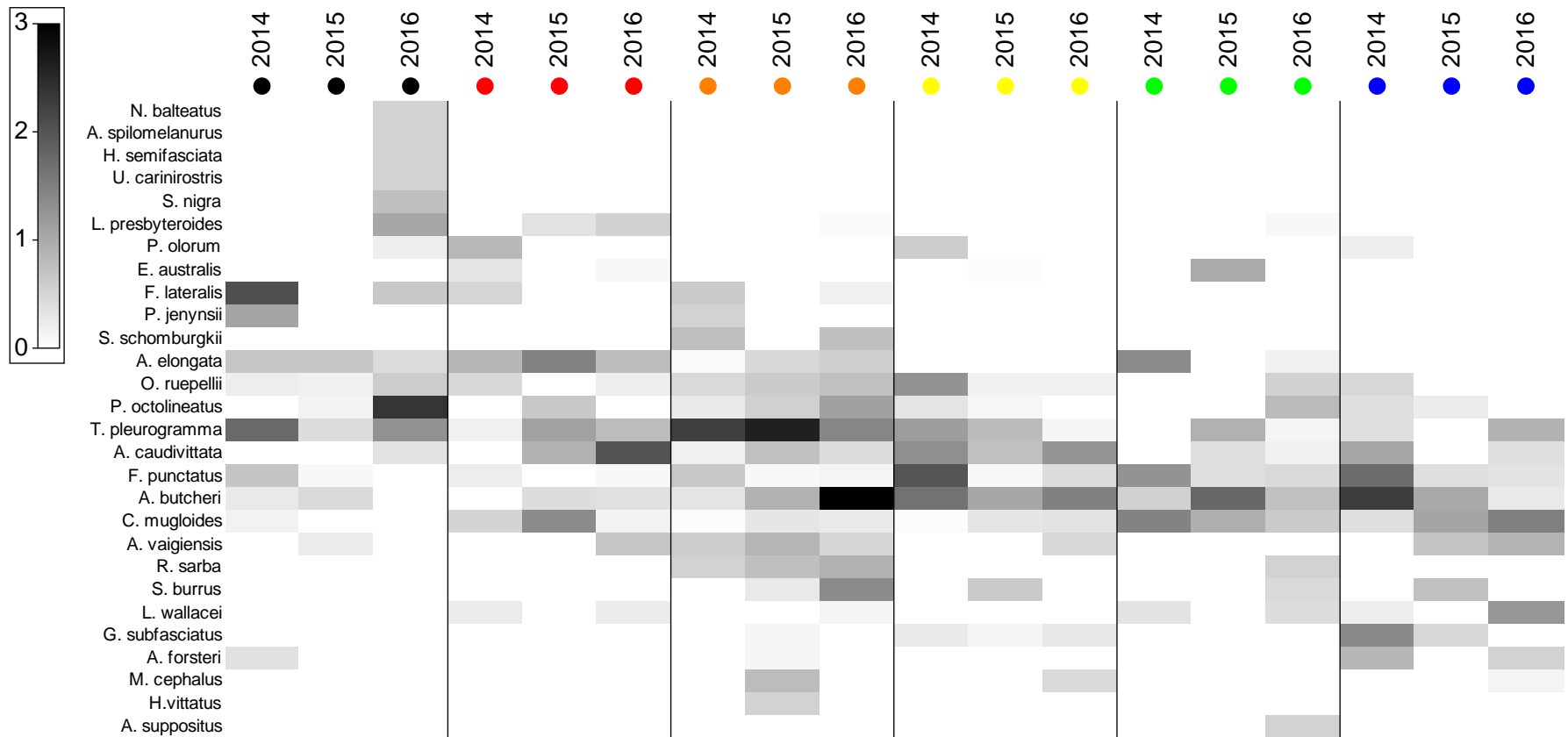


Figure 3.16: Shade plot dispersion weighted and square-root transformed densities of each of the 28 species identified to predate or potentially predate on *M. dalli* in each of the seven regions during each month (November-March) of each year (2013/14, 2014/15 and 2015/16) in the Swan-Canning Estuary. White space denotes the absence of a species, with the grey scale representing the pretreated abundances. Regions; Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●) and the Middle Swan Estuary (●).

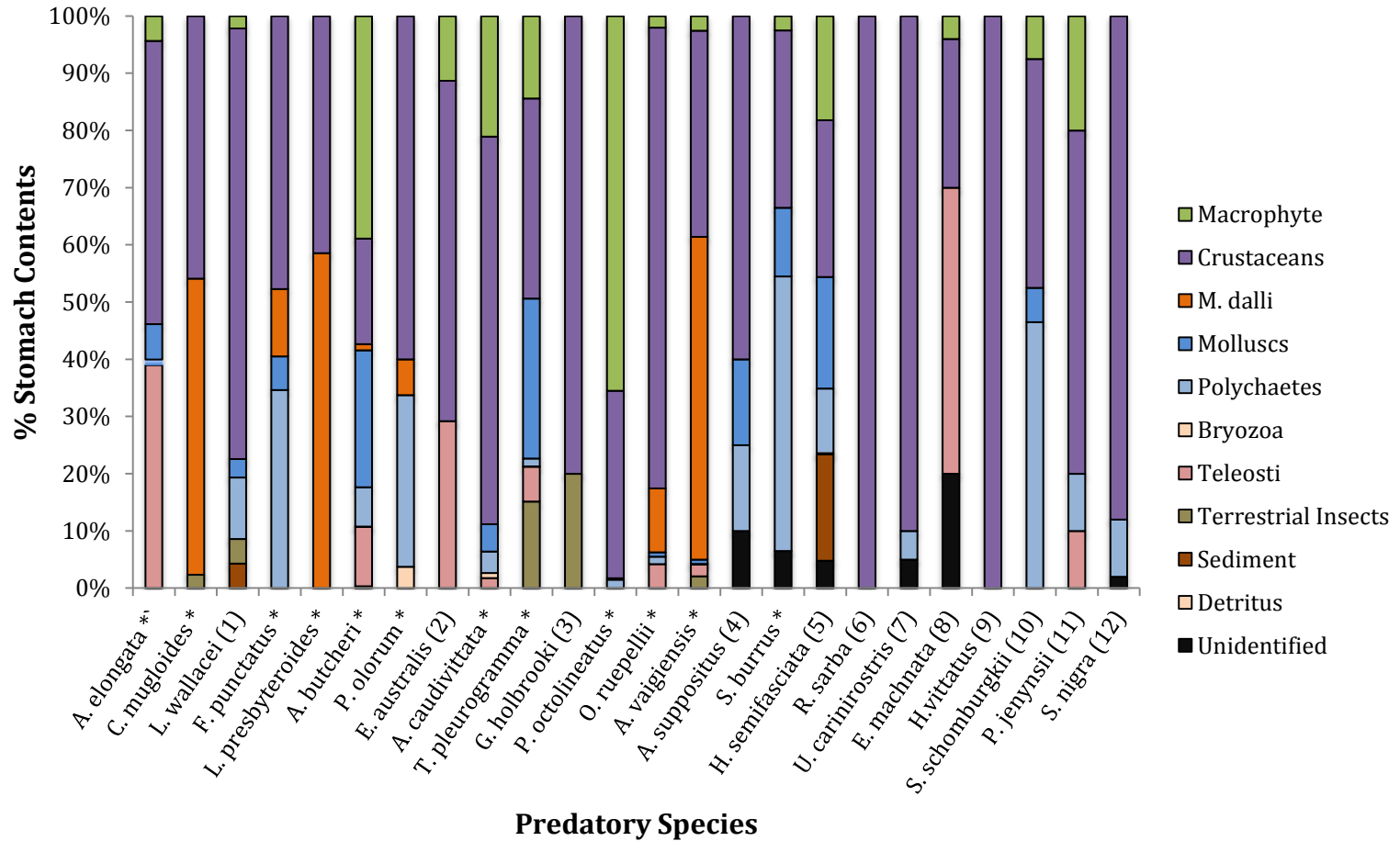


Figure 3.17: Contribution of prey items to the stomachs of the teleost species identified to predate or potentially predate upon *M. dalli*. * indicates those species that were sampled by Poh (unpublished) after the release of *M. dalli* into the area, separating *M. dalli* from other crustaceans in the stomach contents. The numbers represent studies by others that were used to identify the stomach contents of the fish not examined by Poh (unpublished). (1 Humphries and Potter, 1993; 2 MacArthur and Hyndes, 2007; 3 Pen and Potter, 1991; 4 Gaughan and Potter, 1997; 5 MacArthur and Hyndes, 2007; 6 Gaughan and Potter, 1997; 7 Lawson and Aguda, 2010; 8 Dalu *et al.*, 2012; 9 Hyndes *et al.*, 1997; 10 Rodrigues *et al.*, 2012; 11 Lawson and Aguda, 2010, 12 Smith *et al.*, 2011).

3.3.2. SMART results

The following section contains the results of SMART, split into night, day and the day/night comparison model. More detailed results for all variables, *i.e.* scores in each region during each month of each year, are provided as shade plots in the Appendices.

3.3.2.1. Night

Three-way PERMANOVA identified significant differences in SMART score among Region, Month and Year (*i.e.* the breeding season, Nov-March of 2013/14, 2014/15 and 2015/16) and all two way interaction terms (Table 3.4). The mean squares for Region (2,721) was by far the greatest and over five times greater than that for the next most influential terms in the model (*i.e.* Year, 518 and Month, 431). As the proportion of the variance explained by each of the main effects was markedly greater than any of the interaction terms, post-hoc test focused on differences in SMART among Region, Months and Year (Table 3.5).

Table 3.4: Mean squares (MS), pseudo F-ratios (pF) and significance levels (*p*) from a three-way PERMANOVA test on the SMART scores among the seven regions in the Swan-Canning Estuary, between November and March in each of three year. Data obtain during the night. Df = degrees of freedom. Significant results are highlighted in bold.

	Night			
	df	MS	pF	<i>p</i>
Main effects				
Region	6	2721.3	53.38	0.001
Month	4	431.4	8.46	0.001
Year	2	518.3	10.17	0.001
Interactions				
Region x Month	24	83	1.63	0.03
Region x Year	12	123.4	2.42	0.002
Month x Year	5	158.5	3.11	0.01
Region x Month x Year	30	34.4	0.68	0.91
Residual	396	51		

A pairwise PERMANOVA test conducted on the Region main effect, detected significant differences in 18 of the 21 comparisons (Table 3.5a). T-values were greatest for comparison involving the Entrance Channel, which was due to SMART scores for sites in this region being significantly lower (49) than all other regions (58-69; Fig. 3.18). The next highest t-values were found in comparisons involving Perth Water (69) and South Melville Water (58), due to these regions having high and low SMART scores, respectively. The highest scores were found in the Lower Canning Estuary (also 69), and thus statistically similar to those in Perth Water and the Upper Canning Estuary (65).

Although the range of SMART scores among months (*i.e.* 59-63) was less than that among regions (49-69), significant differences were detected among months, with the SMART scores for November and December being different to those in all other months (Table 3.5b). Values in these two months (~63) were higher than that in the remaining months and SMART scores declined progressively from 62 in January to 61 in February and 59 in March (Fig. 3.19). Pairwise PERMANOVA also detected differences in SMART scores among years, with those for 2013/14 (60) being significantly lower than both 2014/15 (63) and 2015/16 (62; Table 3.5c; Fig. 3.20). No significant difference was detected between the last two years.

Table 3.5: T-statistic values derived from a pairwise PERMANOVA tests on the SMART scores for the (a) Region, (b) months and (c) Year main effects. Significant pairwise comparisons are highlighted in grey ($p < 0.05$). EC = Entrance Channel, NMW = North Melville Water, SMW = South Melville Water, PW = Perth Water, LC = Lower Canning, UC = Upper Canning, MS = Middle Swan.

(a) Regions						
Region	EC	NMW	SMW	PW	MS	LC
NMW	10.81					
SMW	6.83	4.74				
PW	13.46	3.17	7.77			
MS	7.59	3.07	1.32	6.33		
LC	13.58	3.87	8.3	0.82	6.77	
UC	12.75	1.99	6.69	1.33	5.3	2.1

(b) Months

Month	Mar	Feb	Jan	Dec
Feb	1.24			
Jan	1.5	0.22		
Dec	4.82	3.44	3.35	
Nov	4.48	3.13	4.02	0.43

(c) Years

Year	3 (2015/16)	2 (2014/15)
2 (2014/15)	0.27	
1 (2013/14)	3.92	3.99

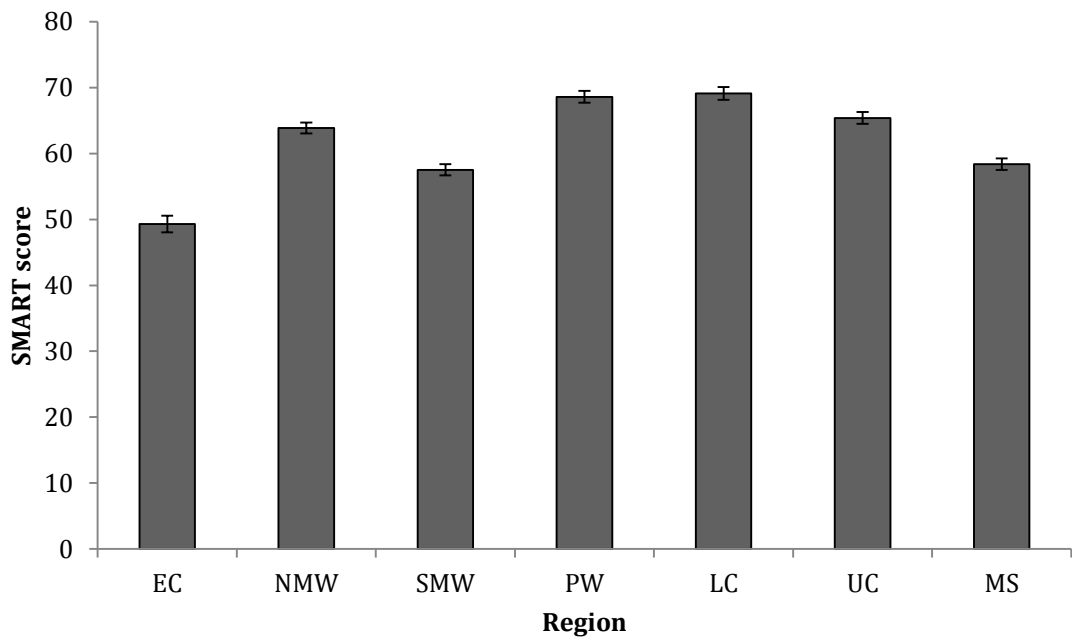


Figure 3.18: Average SMART scores for each of the seven regions in the Swan-Canning Estuary at night (pooled across Month and Year). Error bars represent ± 1 standard error Regions; Entrance Channel (EC), North Melville Water (NMW), South Melville Water (SMW), Perth Water (PW), Lower Canning Estuary (LC), Upper Canning Estuary (UC) and Middle Swan Estuary (MS).

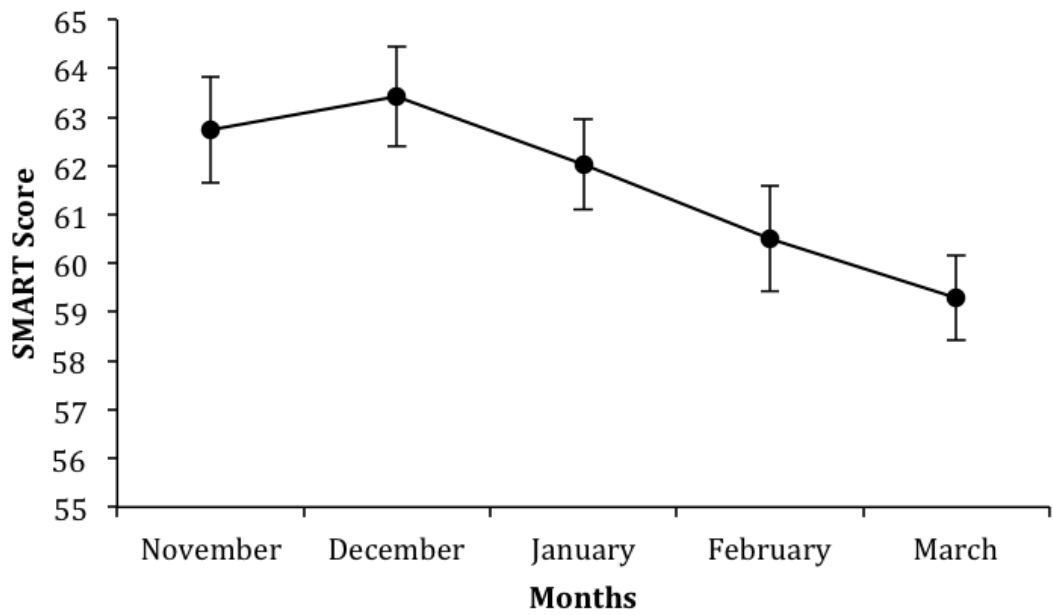


Figure 3.19: Average SMART score at night in the Swan-Canning Estuary in each month between November and March (pooled across Region and Year). Error bars represent ± 1 standard error.

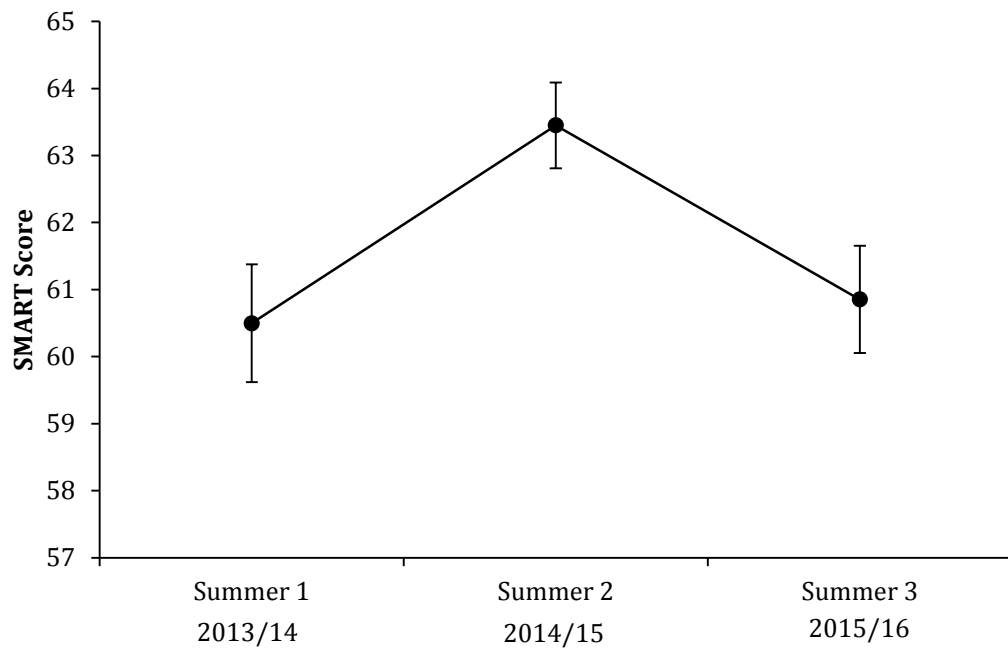


Figure 3.20: Average SMART score at night in the Swan-Canning Estuary in each year between 2013/14 and 2015/16 (pooled across Region and Month). Error bars represent ± 1 standard error.

Shade plots provide a visual indication as to the reason for a high or low overall SMART score as they denote the score of each factor and its component variables. Note that in the case of Region, the shade plot has been constructed at the site level to showcase the variability among sites within a region, as it is at this spatial level that a release strategy would operate.

Among individual sites, the highest scores were recorded at Dalkeith South Perth and Deep Water Point. Each of these sites featured relatively high scores across all factors (Fig. 3.21). In contrast the lowest overall scores for a site was recorded at Stirling Bridge and Leeuwin Barracks and these featured high scores for water quality and predation, however, a very low score for sediment composition and conspecifics.

Shadeplots of the SMART score of the combined regions over months (November – March of each year) showed a relatively similar score over all months, however the makeup of this score from variables differed (Fig. 3.22). For example, January, February and March all had considerably higher scores for water quality than November and December, however, these month had a considerably lower score for predation mainly due to the increase densities of *O. rueppellii*. Not all the factors differed markedly among month, with sediment composition and the densities of competitor and conspecifics remaining fairly similar (Fig. 3.22).

The slightly lower SMART score in 2013/14 than in both 2014/15 and 2015/16 is explained by the fact the water quality measures, particularly salinity, were significantly higher in the last two summers than 2013/14, this is despite the fact that the predation score was slightly higher score in 2013/14, due to the increased densities of *O. rueppellii* in the latter two summers (Fig. 3.23).

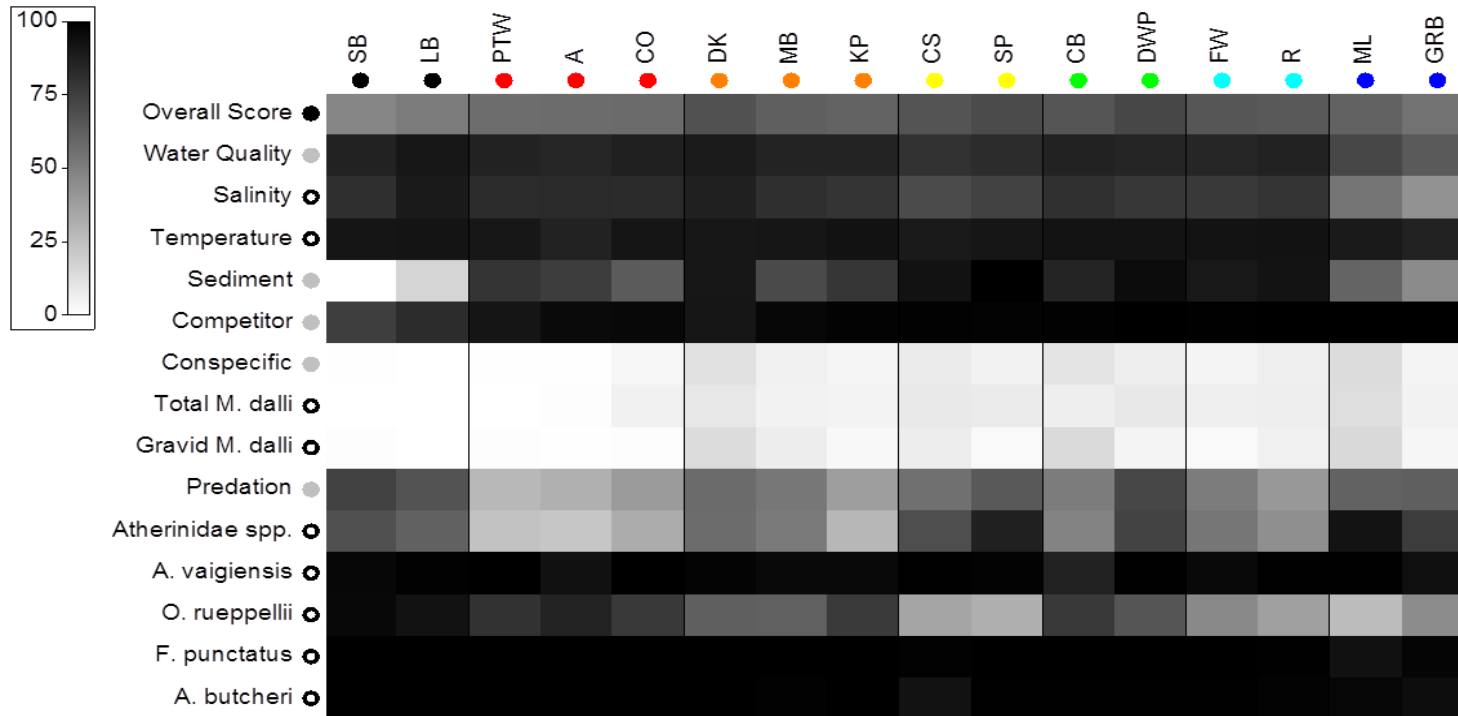


Figure 3.21: Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○), except in the case of the abundances of each predator taxa which have been standardised to place them on a common scale with the other variables, among sites (pooled across Month and Year). The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.

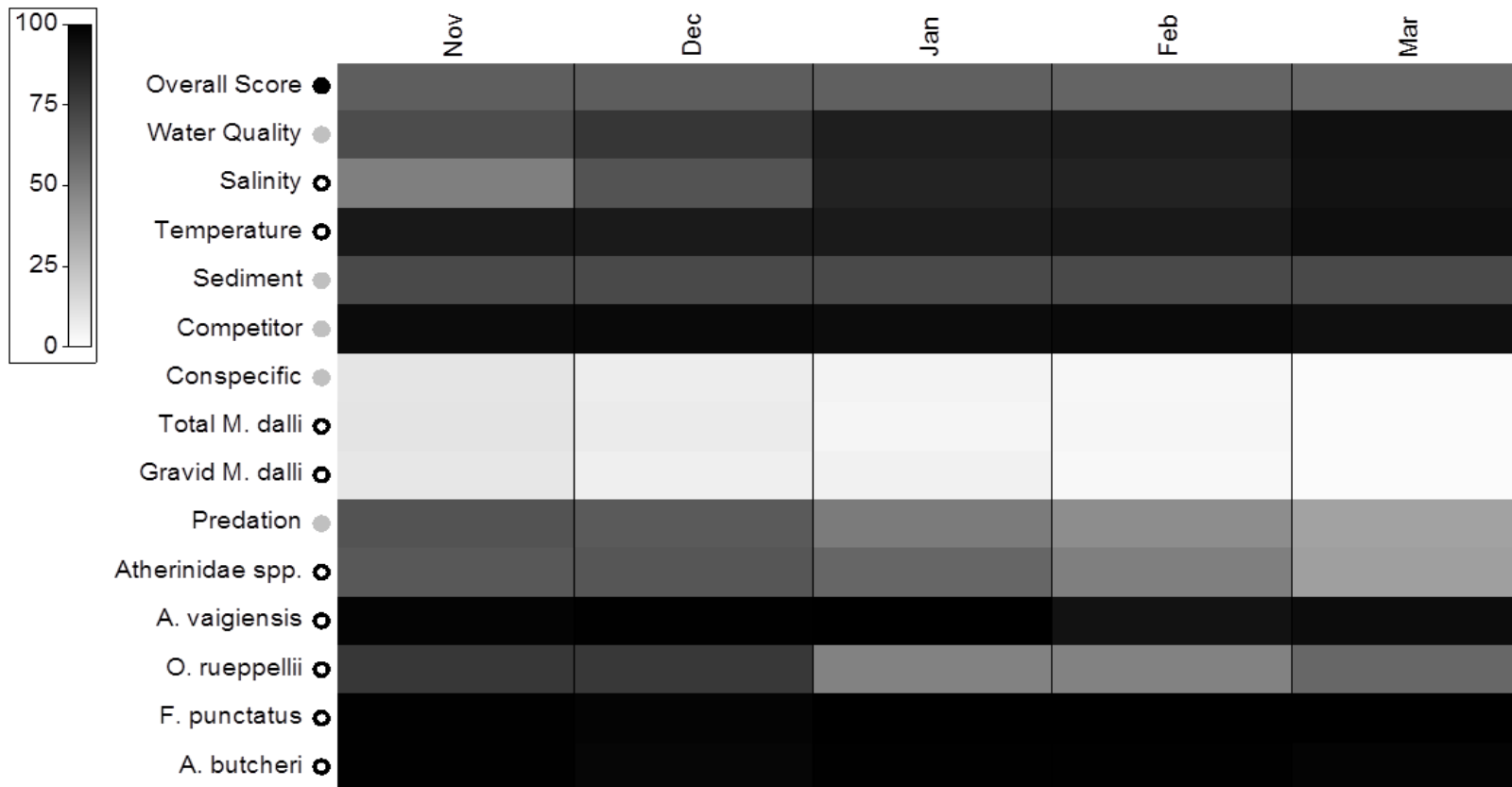


Figure 3.22: Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○), except in the case of the abundances of each predator taxa which have been standardised to place them on a common scale with the other variables, among months (pooled across Region and Year). The greyscale from white to black denotes increasing SMART scores and thus a better release month for hatchery-reared *M. dalli*.

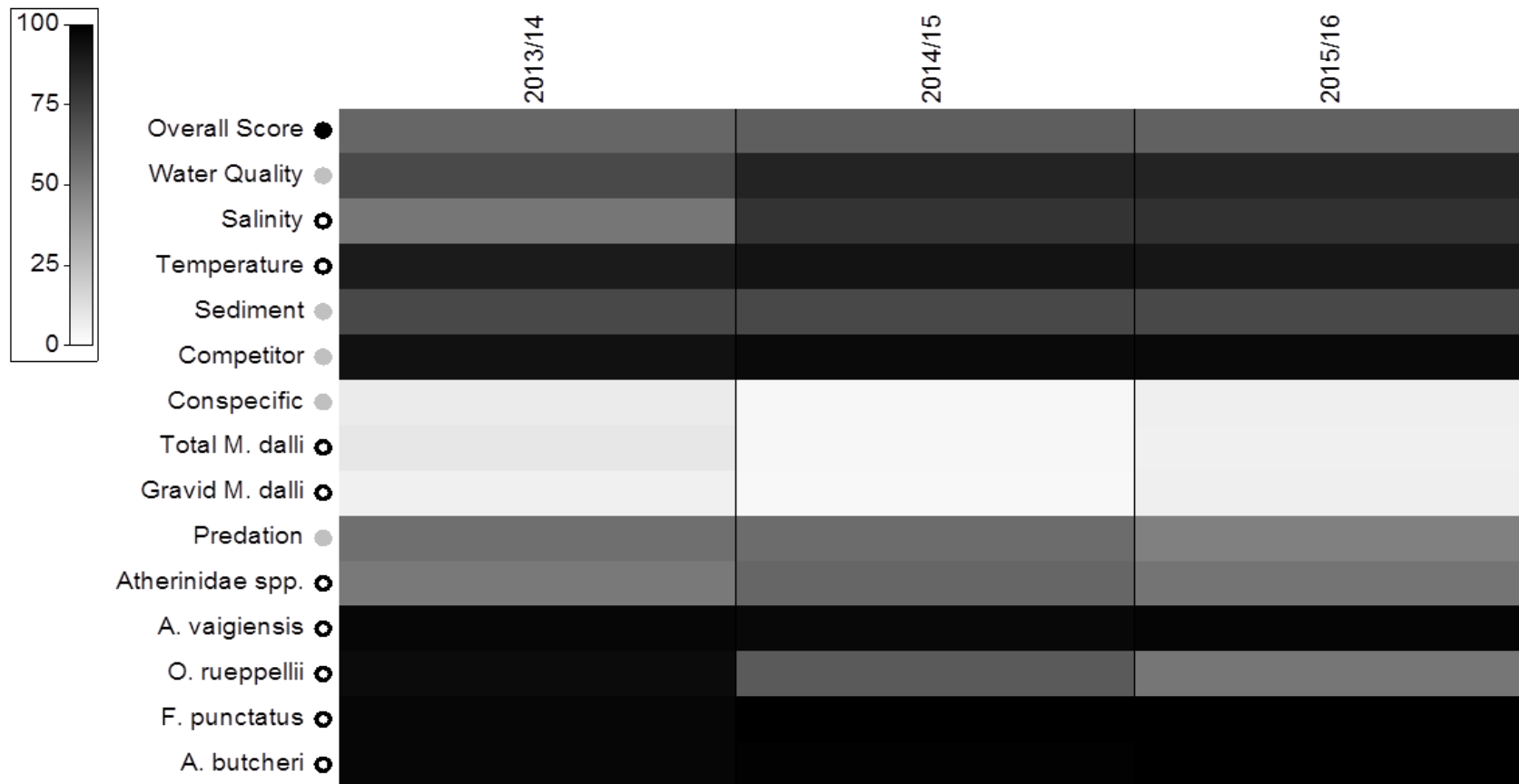


Figure 3.23: Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○), except in the case of the abundances of each predator taxa which have been standardised to place them on a common scale with the other variables, among years (pooled across Region and Month). The greyscale from white to black denotes increasing SMART scores and thus a better release month for hatchery-reared *M. dalli*.

3.3.2.2. Day

A one-way PERMANOVA identified significant differences among regions in SMART score during the day (Table 3.6). Pairwise analysis determined that the biggest difference among regions were comparisons involving the Entrance Channel (Table 3.7), which had the lowest score (25). Sites in Perth Water had the highest score (71), while no differences were detected between North Melville Water, Middle Swan Estuary and the Lower Canning Estuary as their score ranges between 53 and 60 (Fig. 3.24). In general, the average SMART score for each region increased with distance from the estuary mouth from 25 at Entrance Channel to 71 at Perth Water, before declining in the Middle Swan (52, Fig. 3.24).

Table 3.6: Mean squares (MS), pseudo F-ratios (pF) and significance levels (*p*) from a three-way PERMANOVA test on the SMART scores among the six regions in the Swan-Canning Estuary, between November and March in each of three year. Data obtain during the night. Df = degrees of freedom. Significant results are highlighted in bold.

Main effects	Day			
	df	MS	pF	<i>p</i>
Region	5	1400.1	6.8	0.001
Residual	33	206		

Table 3.7: Values of the *T* statistic derived from one-way ANOSIM tests on the SMART score for the regions during the day. Insignificant pairwise comparisons are highlighted in grey (*p* = <0.05). EC = Entrance Channel, NMW = North Melville Water, SMW = South Melville Water, PW = Perth Water, LC = Lower Canning, MS = Middle Swan

Region	EC	MS	PW	SMW	NMW
MS	1.05				
PW	1.5	2.61			
SMW	1.6	0.83	2.82		
NMW	0.88	0.05	2.23	0.76	
LC	4.14	3.94	5.24	2.21	3.31

The overall SMART score differed considerably amongst regions during the day (Fig. 3.25). Windan Bridge had the highest overall SMART score, due to high

scores for sediment composition and predation, as well as relatively high scores for both the water quality variables. The second highest overall score was recorded at Perth Water, which also had a very high sediment composition score, but slightly lower scores for water quality and predation than those at Windan Bridge. Similarly to the night, the lowest score was recorded in the Entrance Channel, with Point Walter recording very low scores for sediment and predation as well as a relatively low score for water quality.

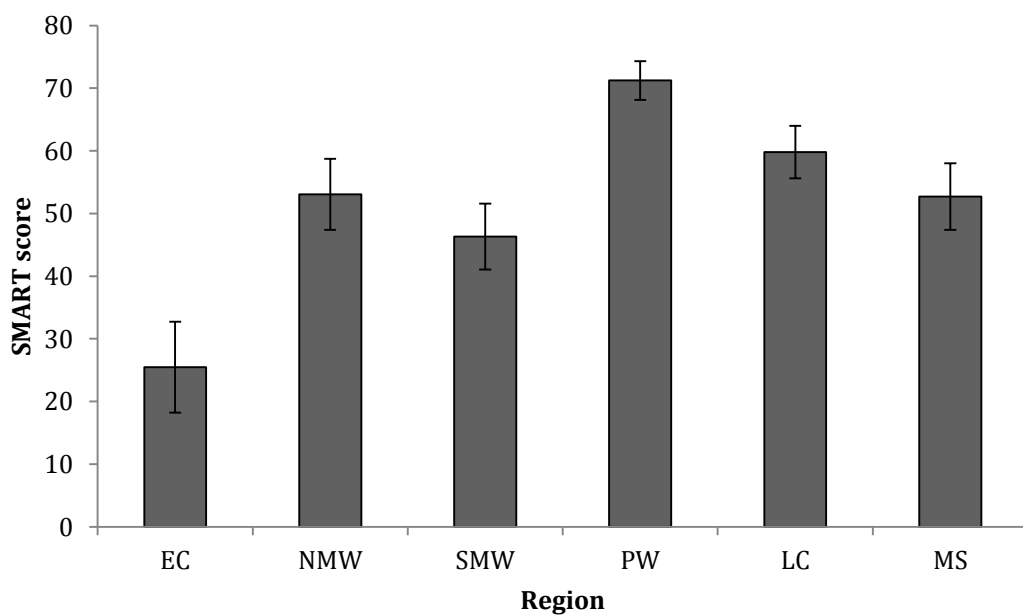


Figure 3.24: Average SMART scores for each of the six regions in the Swan-Canning Estuary at day (pooled across Year). Error bars represent ± 1 standard error Regions; Entrance Channel (EC), North Melville Water (NMW), South Melville Water (SMW), Perth Water (PW), Lower Canning Estuary (LC) and Middle Swan Estuary (MS).

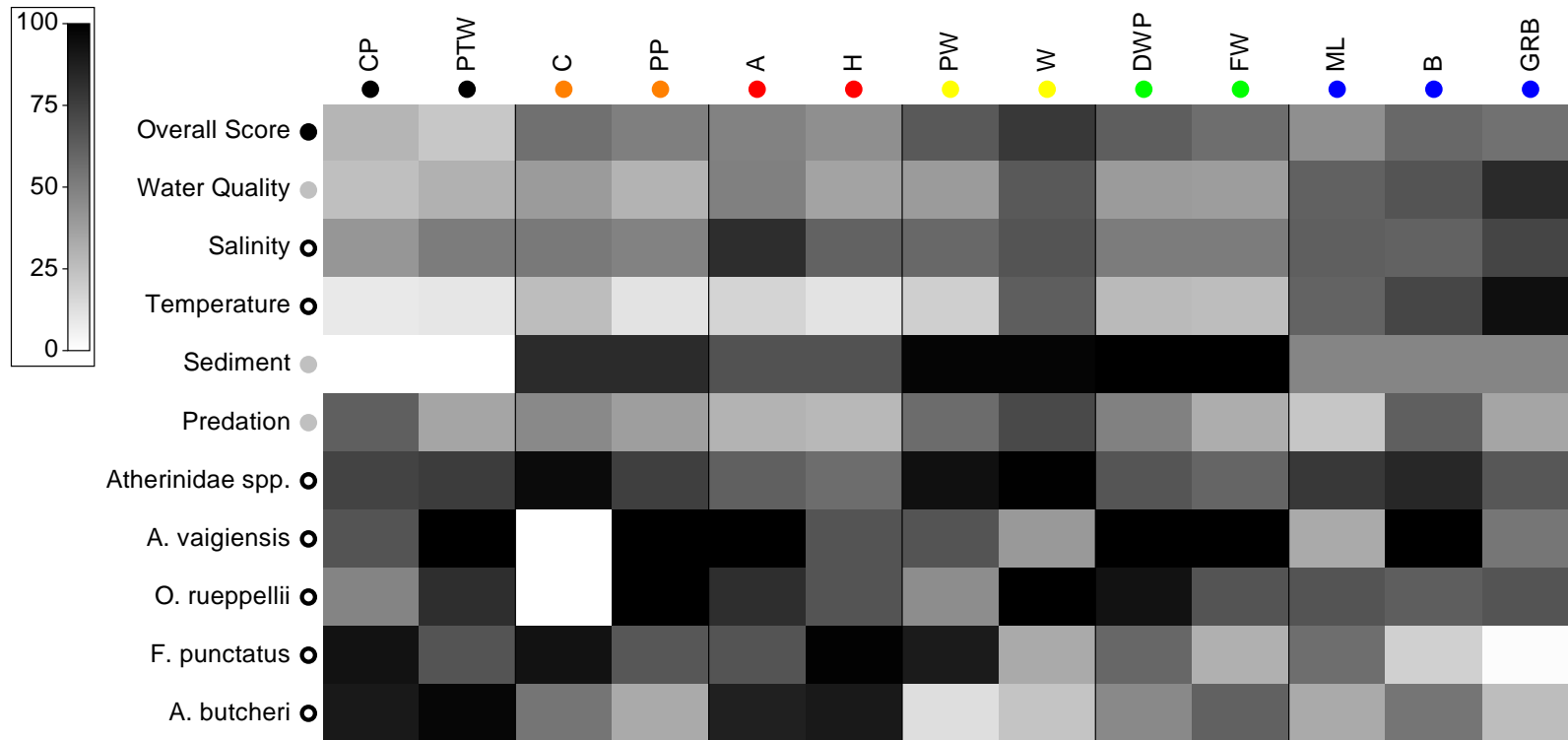


Figure 3.25: Shade plot, constructed using the raw overall SMART scores during the day (●) and the raw scores for each factor (●) and variable (○), except in the case of the abundances of each predator taxa which have been standardised to place them on a common scale with the other variables, among sites (pooled across Year). The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* CP = Chidley Point, PTW = Pt Walter, C = Claremont, PP = Pelican Point, A = Attadale, H = Heathcoate, PW = Perth Water, W = Windan Bridge, DWP = Deep Water Point, FW = Freeway, ML = Maylands, B = Belmont and GRB = Garratt Rd Bridge.

3.3.2.3. Day vs Night

A two-way PERMANOVA detected significant differences in overall SMART scores for both Regions and Day/Night (Table 3.8). The mean squares indicated that the day/night factor (8653.6) explained more than 15 times the variation in the analysis than Region (559.8). Pairwise PERMANOVA among the regions recorded only 4 significant pairwise comparisons of the 15, the largest of which was between South Melville Water and the Lower Canning Estuary (Table 3.9).

Table 3.8: Mean squares (MS), pseudo F-ratios (pF) and significance levels (*p*) from a three-way PERMANOVA test on the SMART scores among the six regions in the Swan-Canning Estuary, between November and March in each of three year. Data obtain during the night. Df = degrees of freedom. Significant results are highlighted in bold.

Main Effects	Day vs night			
	df	MS	pF	<i>p</i>
Region	5	559.76	3.41	0.012
Day/Night	1	8653.6	52.7	0.001
Interactions				
Region x Day/Night	4	168.98	1.03	0.425
Residual	37	164.16		

Table 3.9: Values of the *T* statistic derived from one-way ANOSIM tests on the SMART score for the regions during the day and night combined. Insignificant pairwise comparisons are highlighted in grey. NMW = North Melville Water, PW = Perth Water, LC = Lower Canning, UC = Upper Canning, MS = Middle Swan

Region	NMW	MS	UC	LC	PW
MS	0.35				
UC	0.01	1.69			
LC	1.60	2.43	0.71		
PW	1.64	2.43	0.56	0.08	
SMW	1.11	1.02	1.76	3.18	3.09

Average SMART scores across the day and night for each region indicated a relatively consistent increase in scores with increasing distance upstream (Fig. 3.26). The highest score was recorded in the Middle Swan (73) and the lowest in South Melville Water (53). The average SMART score during the night (81) was almost double that of the average day score (47).

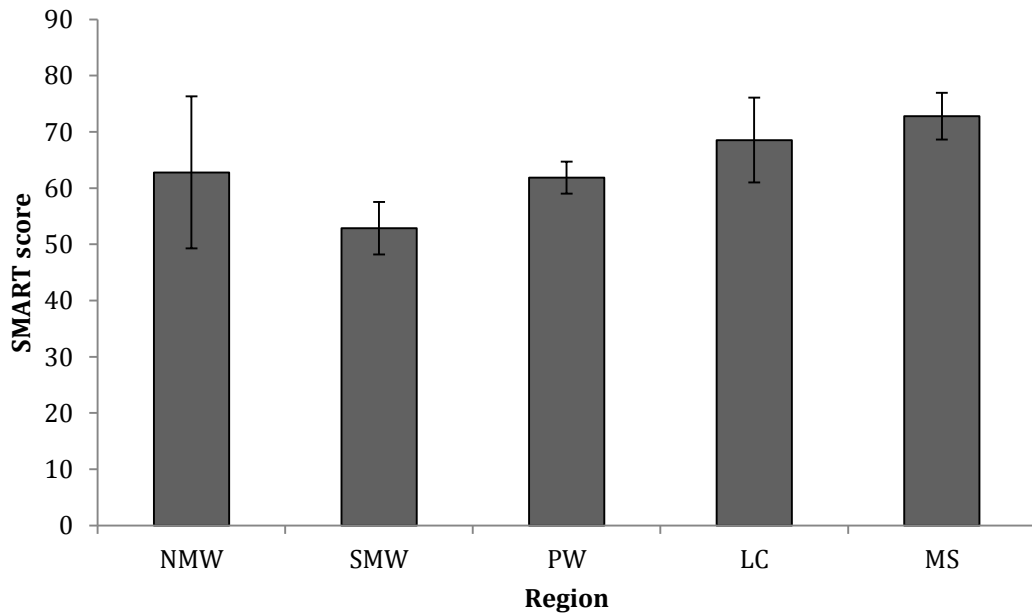


Figure 3.26: Average SMART scores for each of the six regions in the Swan-Canning Estuary during the night and day combined (pooled across each Year). Error bars represent ± 1 standard error Regions; Entrance Channel (EC), North Melville Water (NMW), South Melville Water (SMW), Perth Water (PW), Lower Canning Estuary (LC) and Middle Swan Estuary (MS).

Comparison of sites between day and night, which were solely based on predation, identified that Garratt Road Bridge had the highest SMART score, with excellent scores for three of the five predator variables (species), and moderately high scores for the remaining two, *Atherinidae* spp. and *O. rueppellii* (Fig. 3.27). Deep Water Point had the second highest score overall, however, while it did not get a high score for any of the five predatory variables, each scored highly. The two sites in South Melville Waters, Point Walter and Applecross recorded the lowest overall scores, with relatively low scores across all of the five predator variables (Fig. 3.27).

Comparison across all sites between night and day indicated that night was considerably better in three of the five predatory variables (*A. vaigiensis*, *F. punctatus* and *A. butcheri*, Fig. 3.28). It was also slightly better for the score of the variable *Atherinidae* spp. *Ostorhinchus rueppellii*, however had a considerably lower score for predation during the day than at night.

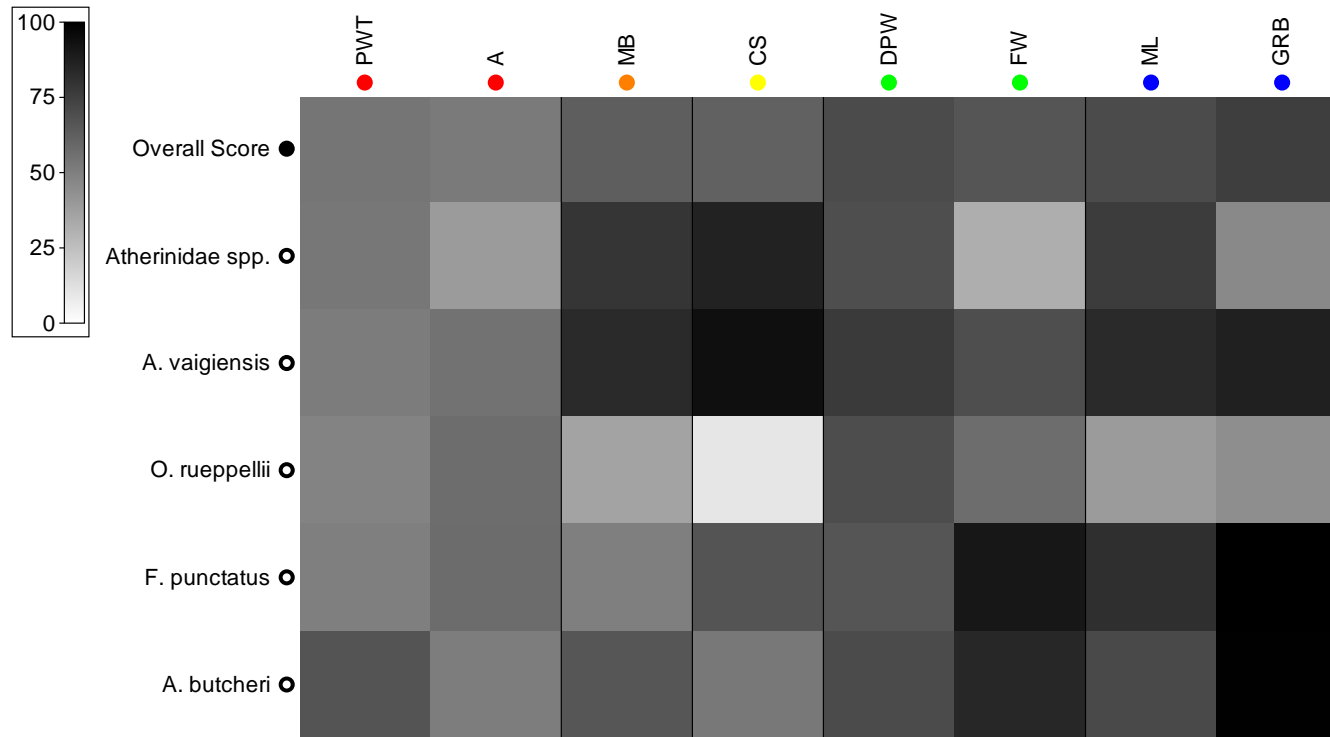


Figure 3.27: Shade plot, constructed using the raw overall SMART score of the day/night comparison (●) and the raw scores for each factor (●) and variable (○), except in the case of the abundances of each predator taxa which have been standardised to place them on a common scale with the other variables, among sites (pooled across Year). The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* CP = Chidley Point, PTW = Pt Walter, C = Claremont, PP = Pelican Point, A = Attadale, H = Heathcoate, PW = Perth Water, W = Windan Bridge, DWP = Deep Water Point, FW = Freeway, ML = Maylands, B = Belmont and GRB = Garratt Rd Bridge.

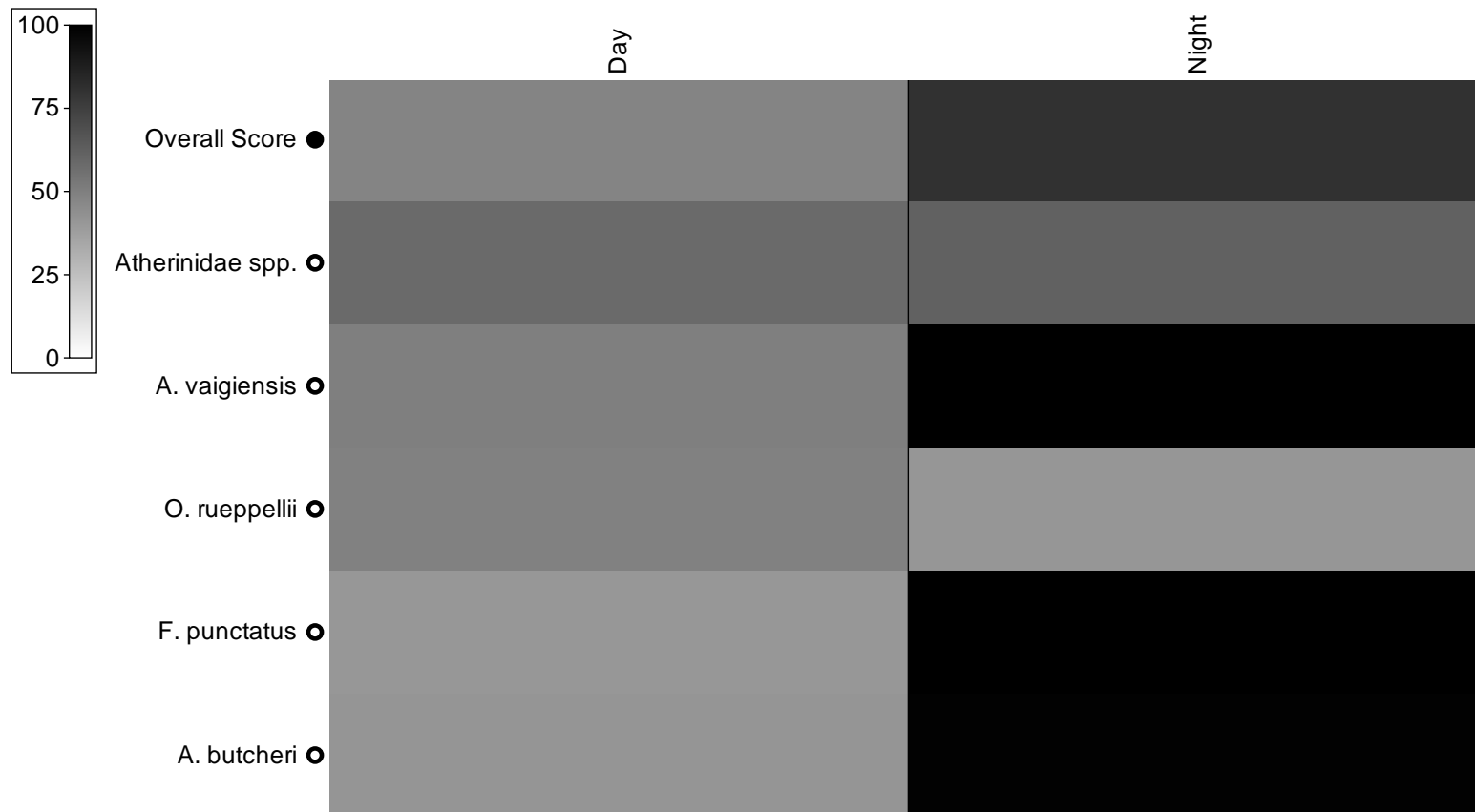


Figure 3.28: Shade plot, constructed using the raw overall SMART score of the day/night comparison (●) and the raw scores for each variable (○) during the day and night. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*.

3.4. Discussion

The primary focus of this chapter was to develop and test a quantitative methodology for evaluating potential sites and times of release for post-larval *Metapenaeus dalli* in the Swan-Canning Estuary. This was achieved examining a suite of abiotic and biotic variables considered as likely to influence the post-release survival of *M. dalli* in the Swan-Canning Estuary using the Survival-Maximisation-At-Release-Tool (SMART). These variables were selected through an extensive literature search and stakeholder engagement and evaluated via the collation of published and unpublished data held at Murdoch University to produce a final SMART score for each site (region) in each month and year (see Appendix 1) as well as a comparison of scores between day and night releases.

The only study found to use a similar tool to that developed here was undertaken to determine suitable sites for the release of the European Wild Rabbit (*Oryctolagus cuniculus*) in the Iberian Peninsula (Carvalho and Gomes, 2003). In that earlier study, the tool focused predominantly on a single variable, habitat, using Geographical Information Systems to select a release site with the highest quantity of optimal habitat within 200 m, *i.e.* the predetermined extent that released *O. cuniculus* travel to settle. Potential release sites were graded from 0 to 100 based on their suitability, with a score of 100 being optimal. The tool developed in this Thesis is much more comprehensive and takes into account a range of environmental and biological variables.

The ensuing discussion has been written in four sections. The first interprets the results of the SMART, focusing on the variation of results during the night, with less comprehensive discussion of the more limited results for the day. The second section evaluates ways in which the tool may be enhanced in the future to better guide the selection of release sites and times of release, followed by a discussion of how the tool may be adapted for the release of other species and other water bodies. The fourth and final section explains how the outputs of the

SMART could be displayed in order to facilitate discussions among different groups in developing an optimal release strategy based on selecting the best sites and times for release.

3.4.1. Selection of best release sites

Statistical analyses of the SMART outputs determined that Region of the Swan-Canning Estuary had the greatest influence on the potential to maximise the post-release survival of hatchery-reared post-larval *M. dalli* during the night. Year (*i.e.* 2013/14, 2014/15 and 2015/16) was the next most influential factor, followed by Month (*i.e.* November-March). Although some of the interactions between release Region, Year and Month were also significant, they accounted for a far smaller proportion of the variation in the SMART scores any of the main effects, and particularly that of Region. Combining each site, month and year, the best release site and time for juvenile *M. dalli* in the Swan-Canning Estuary was Deep Water Point in the Lower Canning Estuary at night in January 2014 (SMART Score = 80, Appendix 5). This site had high scores for water quality, sediment composition, low abundances of competitor and predatory species and relatively high scores for the abundance of conspecifics.

3.4.1.1. Regional differences

Across all months and years, the best regions for release were the Lower Canning Estuary and Perth Water, with the former region having the highest average SMART score across the estuary. Both of these regions scored highly for water quality, sediment composition and competitors, as well as receiving relatively high scores for conspecifics and predation. Variance in water quality scores was mainly due to the influence of salinity, with very high to optimal scores for water temperature recorded throughout much of the estuary. Perth Water had a lower overall water quality score than the Lower Canning Estuary, probably due to the slightly lower salinity in the former region, which is due to the catchment of

the Swan River being much larger than that of the Canning and the fact that stop boards are placed into Kent Street Weir between September/October, thus preventing freshwater discharge entering the Canning axis of the estuary (Swan River Trust, 2009). As the optimal value of salinity used to determine SMART score in this experiment is close to that of full strength seawater (Crisp *et al.*, 2017), the fresher nearshore waters of the Perth Water region are less suitable for the release of *M. dalli*, explaining the lower score for salinity than that recorded in the Lower Canning Estuary.

While scores for sediment composition, competitors and conspecifics were similar for the two regions, those for predation differed. Predation scores for *A. vaigiensis*, *F. punctatus* and *A. butcheri* were very high in both of the Lower Canning Estuary and Perth Water, however, scores for atherinids and *O. rueppellii* varied. Predation by atherinids was the most influential on the overall predation score in the Lower Canning Estuary. Atherinids were present in this region throughout the sampling period, a trend consistent with studies on the fish assemblages of the Swan-Canning Estuary by Loneragan and Potter (1990), in which it was observed that the atherinids *A. elongata* and *C. mugiloides* dominated percentage contribution to the overall density of fish in the Melville Waters and Lower Canning Estuary. *Ostorhinchus rueppellii* was observed in high abundance in the Perth Waters across the sampling period, consistent with observations by Loneragan and Potter (1990). This apogonid, which is classified as a marine and estuarine species (Potter *et al.*, 2015a), migrates to the shallows of the upper estuary during the early summer to spawn (Chrystal *et al.*, 1985), explaining the low scores for predation by this species in the summer months.

The Entrance Channel was identified as being the worst region for the release of post-larval *M. dalli*. While this region received high scores for water quality and predation, it had very low scores for sediment composition and conspecifics as well as the lowest score for competitors. The inorganic portion of

the sediment in this region were typical of those recorded in nearshore coastal waters of south-western Australia (Wildsmith *et al.*, 2005), with grain dominated by the ~125-249 μm size fraction, smaller than that observed in most other regions throughout the estuary and considered optimal for *M. dalli*, *i.e.* 249-500 μm (Bennett, 2014). Moreover, this region, likely due to its seagrass beds, had the largest amount of particulate organic matter, which inhibits burying in small *M. dalli* (Bennett, 2014). Sites in the Entrance Channel received very low scores for conspecifics due to the low numbers of adult and gravid *M. dalli*. This trend is consistent with observations of *M. dalli* abundance between 1977 and 1982, where this species was mainly recorded from North Melville Water and further upstream, which was attributed to the annual migration of adult *M. dalli* from the deeper, offshore waters of the estuary into the shallow waters of the upstream regions to spawn in early summer (Potter *et al.*, 1986). This trend is also consistent with patterns in distribution of *M. dalli* in summer in the Peel-Harvey Estuary, 100 km south of the Swan-Canning Estuary, prior to the construction of the Dawesville Cut (Potter *et al.*, 1989).

Relatively large densities of *Penaeus latisulcatus* in this region resulted in the Entrance Channel receiving a low score for competitors. This penaeid is regarded as a marine estuarine-opportunist (Potter *et al.*, 2015b) and has a lifecycle similar to many other marine penaeids, spawning in the marine environment and post-larvae/juveniles recruiting to sheltered coastal environments and/or estuaries (Dall, 1990; Potter *et al.*, 1991; Bailey-Brock and Moss, 1992). This life history strategy, the abundance of suitable habitat and the maintenance of high salinities year round in the lowermost regions of the Swan-Canning Estuary are likely to be the key factors limiting the distribution of *P. latisulcatus* predominantly to the Entrance Channel, and thus explaining why all other regions recorded very high scores for competitors.

3.4.1.2. Interannual differences

Although variation in SMART score across years was low in comparison to that across regions, significant differences were detected. The highest average SMART score was recorded in 2014/15, due to higher scores for water quality and predation than those in the other two years. Scores for all other factors were similar across the three years. Variation in the predation across all three years was predominantly due to the scores for atherinids and *O. rueppellii*. Scores for *O. rueppellii* were highest during 2013/14, likely due to the lower salinity precluding the migration of this apogonid from the more saline offshore waters into the nearshore areas of the estuary.

3.4.1.3. Monthly differences

Although the overall SMART scores were relatively consistent across months, scores for the factors of each month varied considerably. For example, water quality scores were lowest in November and December, due to the lingering influence of freshwater discharge from the upper river in late spring/ early summer. Discharge decreases as summer progresses, leading to the intrusion of saltwater further upstream (Tweedley *et al.*, 2016a; Broadley *et al.*, 2017), resulting in salinities across the estuary becoming closer to full strength seawater and ideal for the release of *M. dalli* (Thompson, 2001; Crisp *et al.*, 2017). Score for conspecifics (both total and gravid *M. dalli*) were highest in November and decreased progressively throughout summer, likely attributable the movement of individuals back to offshore waters after spawning and, in the case of females, mortality (Potter *et al.*, 1986; 1989; Broadley *et al.*, 2017). Similarly, predation scores were highest in November and December, before decreasing gradually and sequentially over summer. This variation in predation score was influenced by changes in the abundances of atherinids. These species, like most estuarine residents in the Swan-Canning Estuary, spawn in the early summer, *i.e.* December

(Prince and Potter, 1983), resulting in the subsequent recruitment of juveniles increasing the risk of predation on post-larval *M. dalli* as summer progresses.

3.4.1.4. Daytime scores

In considering the more limited information available for the calculation of SMART during the day, Region was the only influential factor. Perth Water received the greatest score, due to good water quality and low levels of predation. Variation in water temperature was much higher during the day than observed during the night, resulting in large variations in water quality score among regions. Daytime water temperatures were much higher in the lowermost regions, reaching ~30 °C and lowering the score for this variable due to it negatively affecting the survival of *M. dalli* (Crisp *et al.*, 2017).

Each of the species incorporated into the predation factor varied markedly across regions, with no clear pattern. In the case of *A. vaigiensis*, *F. punctatus* and *A. butcheri*, the level of variation during the day was far greater than that recorded at night. Ichthyofaunal assemblages in estuaries are very dynamic and can change spatially over a range of temporal scales, including diel phase (Gray *et al.*, 1998; Hoeksema and Potter, 2006). It is relevant that some species undergo diel movements between the shallow, nearshore and deeper offshore waters, with the migrations due to changes in the abundance of fish and avian predators and for feeding (Miller, 1979; Helfman, 1993). In this case, the abundance of predators, increased during the day, which is the opposite trend to that recorded by Hoeksema and Potter (2006) in the upper parts of the Swan Estuary, but the same as those recorded by Yeoh *et al.* (In press) in the Walpole-Nornalup Estuary, which suggests that diel patterns in the movements of atherinids and gobids may be plastic.

The comparison of sites where data were available for both day and night showed that night rather than day releases were more favourable. Scores for four out of the five predator species, *i.e.* all except *O. rueppellii*, were lower at night.

These results contradict the pattern expected based on an empirical study of the predation by teleosts on hatchery-reared *M. dalli* (B. Poh, Murdoch University, unpublished data). That study showed that, while the volumetric contribution of post-larval *M. dalli* to the gut contents of *A. vaigiensis*, *F. punctatus* and *A. butcheri* did not differ markedly between day and night, the mean number of post-larval *M. dalli* ingested by *O. rueppellii* was approximately four times greater during the night than day, the converse of the SMART results. This difference is likely based on the method of identifying predation potential, the SMART scores being based on predator abundance and Poh's calculation involving multiplying the mean number of prawns in stomachs of each species by mean abundance of that species.

3.4.2. Improvements to the SMART

As the SMART is the first objective and quantitative tool developed to combine multiple variables considered to affect the selection of release site for a hatchery-reared population, and it has been developed over a relatively short time period, there are a number of improvements that can be made. Through the first run of the SMART, four areas for improvement became apparent. Firstly, the inclusion of optimal release size and density for release of *M. dalli* into the tool. Secondly, revision of the variables to be incorporated into the tool and the standardisation of these variables onto a single scale. Thirdly, the procedure for weighting and scaling of variables and factors when determining the score for the factors and final SMART scores, and finally, a robust day time sampling regime, similar to that of the night, is required to make more rigorous comparisons of day and night releases.

Variables and standardisation

The variables selected for the model were chosen based on an extensive survey of the literature on studies of survival at release sites and times (Chapter 2), and through discussions with staff at Murdoch University, Department of Parks

and Wildlife, Department of Fisheries WA, Australian Centre for Applied Aquaculture Research and Department of Water. Each variable selected for the inclusion into model was one considered to have a significant effect on the post-release survival of *M. dalli*.

In order to increase the effectiveness of the SMART, some variables require further study to better understand either the range of their values throughout the estuary or their effect on *M. dalli*. For example, it was assumed that the effects of water temperature and salinity on post-larval *M. dalli* are similar to those on post-larval *M. dalli* (Crisp *et al.*, 2017). However, studies on other penaeids suggest that this may not be the case. For example, whilst changes in salinity had an adverse effect on the survival of larval Brown Shrimp *Farfantepenaeus aztecus* (Saoud and Davies, 2003), in studies on post-larval *F. aztecus*, survival over 24 h was not impacted over a wide range of water temperature and salinities. Moreover, post-larval survival in *F. aztecus* was still high after 28 days in a range of water temperature and salinity conditions (Zein-Elden and Aldrich, 1965; Zein-Elden and Renaud, 1986). A wide tolerance in salinity, *i.e.* 1-40, was also recorded in post-larval Northern White Shrimp *Litopenaeus setiferus* and four other species of prawns from Mexico (Mair, 1982). While these studies found that prawns could survive in a broad range of water temperatures and salinities, it was noted that growth, behaviour and function were not optimal across the whole range, which could increase their predation risk. Further study on the effect of salinity and water temperature on post-larval *M. dalli* is required to determine the optimal releases conditions for this stage in the life-cycle and whether it differs from that of the larvae.

More extensive and detailed sediment data would improve the resolution of SMART. Although the sediment data collected by Bennett (2014) is the most in-depth to date, it was based on only two replicates per site, did not cover a sufficiently large number of sites in the different regions and did not study

sediment changes in these regions over a long period of time. Thus, to determine how sediment composition changes spatially and temporally in the Swan-Canning Estuary and the effect this has on *M. dalli*, a more robust sampling regime addressing these issues is required. In the current iteration of SMART, the sediment composition factor is based on a single variable, *i.e.* the PC1 score, despite the fact that data on the contribution of individual grain size fractions were available. These were not used as laboratory studies had not been conducted on the preference of *M. dalli*, and particularly post-larval individuals, to sediments comprised of a single grain size. Moreover, as the Swan-Canning Estuary has been exposed to large amounts of anthropogenic activity over a substantial period of time, the sediments in some areas may contain a range of contaminants, which could potentially affect the post-release survival of *M. dalli*. Whether nutrients, *e.g.* run off from fertilisers, or non-nutrient, *e.g.* leaching of metals from landfill site, the effect of contaminants could be quantified and included in the model, provided laboratory studies and sediment contaminant data were also available. The provision of these data is important when undertaking aquaculture-based enhancements in highly urbanised area, such as estuaries which can be heavily degraded (Jackson *et al.*, 2001; Tweedley *et al.*, 2015).

Further examination of the methodology for standardisation of the scores within the SMART would also be beneficial, *e.g.* the Relative Predation Index (RPI), which was calculated by multiplying the density of a species by its predation score. The aim of developing the RPI was to quantify the level at which a species predated on released *M. dalli* and to remove the bias of abundance of a species when determining the score for the predation variable. This was mainly done to scale down the predation effect of atherinids, which are extremely numerous and scale up that of *O. rueppellii*, which occur in far lower densities. Whilst stomach content analyses identified atherinids to predate predominantly on released *M. dalli* and other small crustaceans, this was based on a small proportion of these

fish containing items in their stomachs (*i.e.* ~28% of *A. elongata*, ~38% of *C. mugiloides* and ~11% of *L. presbyteroides*; Poh, B. Murdoch University, unpublished data). Thus, the predation potential of atherinids was assigned a much lower predation score than that of *O. rueppellii*, which had a much greater percentage of full guts (~76%) which also consisted of greater numbers of released *M. dalli*, with up to 300 found in a single fish stomach. The current scoring of predation may underestimate the predation by *O. rueppellii* and should be reviewed to ensure that the predation potential of each predatory species is accurately accounted for in the tool once the dietary studies of Poh have been completed.

Enhancing the standardisation of scores for the conspecifics and competitor factors would also be valuable for enhancing SMART. Neither of these variables varied greatly in SMART scores and therefore did not have a large influence on the overall score, even though they were identified as being important to the selection of release site and time for juvenile *M. dalli* (Chapter 2). Further exploration of the effect of these variables is required to effectively determine the extent to which abundance of *M. dalli* or *P. latisulcatus* effect post-release survival of juvenile *M. dalli*. This would be enhanced by empirical experimental studies, similar to those carried out on the Eastern King Prawn *Penaeus plebejus* (Ochwada-Doyle *et al.*, 2012) and Brown Tiger Prawn *Penaeus esculentus* (Loneragan *et al.*, 2001).

Scaling and weighting of factors and variables

Where factors were comprised of more than one variable, *e.g.* water quality or conspecifics, both of which had two variables, each variable was given an equal weighting, except for predation where the RPI of each species was added together and the total standardised. The equal weighting of variables in a factor may dampen variation in the overall factor score, as seen for example in the water quality factor where temperature was similar across all sites, but salinity varied. As a result, a positive score for temperature accounted for 50% of the weighting in

the water quality factor, which reduced the effect that variation in salinity had on the overall score and made differentiation between sites less pronounced. Differential weighting of factors, such as temperature and salinity, so that that variation in one, in this case salinity, has more influence on the final score could yield better results. In the case of conspecifics, both total *M. dalli* and gravid *M. dalli* scores were assigned the same weight. However, as the tool was interested mostly in identifying the spawning grounds of the *M. dalli* population, a better approach may be to weight the score for the abundance of gravid female *M. dalli* more than the total abundance.

In the current version of the SMART, each factor was given equal weighting for contribution to the final score, which may not be representative of their importance on post-release survival. For example, predation is often the single greatest obstacle for short-term post-release survival of hatchery-reared juveniles in the wild (Hines *et al.*, 2008; Støttrup *et al.*, 2008). It may therefore be more realistic to weight predation more highly than the other factors in the SMART. Further study is required to determine the relative effect of each of the factors on the selection of release site and time in order to understand the most effective procedure for weighting factors in the tool.

Improvement of the day output

The data available for assessing release sites and times during the day was considerably less comprehensive than that for release at night. The original objective of this Thesis was to determine optimal sites and times for release during the night, as this was when data on the prawn project were collected. However, as some limited data were available for the day from the Fish Community Index sampling program (Hallett and Tweedley, 2014, 2015; Hallett, 2016), the tool was run for the day as well. As the behaviour of prawns differs markedly during the night and day (*e.g.* Wickham and Minkler, 1975; Hill, 1985; Dall, 1990; Wassenberg and Hill, 1994), a more robust sampling regime during the day is required in order

to explore the effect of each variable on prawns at this time. This would lead to a more reliable selection of release sites during the day and vastly improve the comparison of *M. dalli* post-release survival during the night and day.

3.4.3. Adaptation of the SMART to other release programs

The main objective of this Thesis was to use the SMART to determine the optimal release site and time for *M. dalli* in the Swan-Canning Estuary. However, the aim of designing the SMART was to develop a tool that could be adapted for other release programs, *i.e.* for other species in different aquatic systems. Many ongoing release programs include a robust post-release monitoring regime, therefore sufficient data is likely to be available for these programs to modify the SMART for their use and assist in optimising their release strategy. In order to adapt the SMART to be applied to evaluate release strategies for other species and environments, the factors or variables included in the calculation need to be re-evaluated in consultation with the stakeholders for the release. These stakeholders should include researchers/aquaculturists, managers, beneficiaries of the release and the broader community. The following section explores the variables that may need to be included, removed or altered in order to adapt the SMART for selection of a release site for other species.

Water quality

In our study the only water quality variables identified as having a large impact on the survival of released *M. dalli* were water temperature and salinity. Other environmental variables may have an effect on other species and/or on the water body they are being released into. For example, pH, although not included in our study, may have a significant effect on the release of molluscs. Exposure of molluscs to even slightly acidic pH can result in shell dissolution, which may decrease shell strength and thus increase predation on these organisms (Gazeau *et al.*, 2013). The abundance of phytoplankton as a food source may also be a

significant factor for releases of filter feeding organisms such as bivalves (Arapov *et al.*, 2010). While the concentrations of dissolved oxygen were not low during our study in the Swan-Canning Estuary, they have been in the past (*e.g.* Tweedley *et al.*, 2016b) and have the potential to kill large number of released (and wild) individuals. Therefore, dissolved oxygen concentration should be included in future SMART models, and particularly for release strategies in water bodies where periodic hypoxia and anoxic conditions may occur.

Availability of habitat

Sediment composition is an important habitat factor for benthic species, such as *M. dalli*, but is less important for pelagic species. It is therefore necessary to identify habitat preferences or requirements specific to the target species and to include the relevant variable. For example, a study on the habitat preference of juvenile Mulloway *Argyrosomus japonicas* determined that the presence of deep holes influenced the residence times and dispersal of released fish; with individuals' resident for much longer in deeper holes than in shallower release sites (Taylor *et al.*, 2006). Therefore, the presence of, or distance to deep holes, should be included as a variable to select an appropriate release site for *A. japonicas*. For the release of *P. esculentus* in Exmouth Gulf, Loneragan *et al.* (2004) determined that a density of at least 5 gm⁻² of seagrass bed was needed for juvenile settlement, survival and growth. Modeling results based on empirical studies (Haywood *et al.*, 1995; Loneragan *et al.*, 1998; 2001) predicted that much greater numbers of prawns survive in high biomass seagrass beds than those with low biomass or bare substratum (Loneragan *et al.*, 2006). Therefore, seagrass density at each site would be an important factor to include if adapting the SMART for the release of *P. esculentus* in the Exmouth Gulf.

Abundance of competitors

In this study, the only competitor identified as having a potential negative effect on *M. dalli* survival post-release was *P. latisulcatus*. However, the number of competitor species may be larger or the distribution of competitors throughout the estuary may be higher for the release of other species or in other water bodies. For example, competition for space can clearly play a pivotal role in the survival of a species in rocky intertidal communities (Cornell, 1961; Paine, 1966). Whilst *M. dalli* and *P. latisulcatus* showed a high degree of spatial segregation in the Swan-Canning Estuary, teleosts may have a lesser degree of segregation due to their greater movement ability. A possible example of interspecific competition between fish is shown by the release of Brook Trout *Salvelinus fontinalis* in lakes across the Laurentian Shield, Canada. The growth and yield of Brook Trout were inversely correlated with the density of White Sucker *Catostomus commersonii* (Tremblay, 1991), suggesting that interspecific competition may have been having affected trout growth.

Abundance of conspecifics

The presence of conspecifics was considered as positive metric for the release of *M. dalli*. However, this may not be the case for all species and thus it is important to understand the lifecycle of the target species and density-dependent response to determine whether conspecifics have a positive or negative influence on the release. For example, sites where adults are present may not be the best place to release juveniles of all species. If releasing juvenile *P. latisulcatus*, a species that spawns in marine waters and the post-larvae migrate to inshore waters or estuaries, releasing juveniles where adults are found is unlikely to be successful. In eastern Australia, releases of post-larval *P. plebejus* have been made in shallow lagoons distant from the oceanic breeding grounds of this species (Ochwada-Doyle *et al.*, 2009; 2012; Taylor and Ko, 2011). If the wild population of the target species is large, density-dependent effects on growth and mortality may become important

(Lorenzen, 2005). The abundance of conspecifics may also become a negative factor if the carrying capacity of the site is reached, with larger adults outcompeting smaller released individuals. It is unlikely, however, that release of hatchery-reared individuals would be required in such a situation. The point at which density-dependent effects is an important consideration for establishing release densities. For example, field experiments demonstrated the growth of small juvenile Green Tiger Prawns *Penaeus semisulcatus* is not adversely affected until the stocking density exceeds 10 prawns m⁻², a density much greater than high natural densities of 1 to 2 prawns m⁻² in high biomass seagrass beds (Loneragan *et al.*, 2001). Cannibalism by conspecifics may also become a factor. For example, juvenile Blue Crabs *Canninectes sapidus* are cannibalised by adults and peak mortality coincides with the maximum abundance of adults (Zmora *et al.*, 2005; Zohar *et al.*, 2008). Thus, releasing juveniles in areas with high adult abundance may therefore be a negative for releases of this species (Hines and Ruiz, 1995; Johnson *et al.*, 2008).

Prey abundance

The abundance of prey was not included in the SMART as little is known of the diet of *M. dalli*, though they are thought to operate low in the food web, *i.e.* mainly primary consumers and detritivores. For release of predatory teleosts such as *A. japonicus* or Barramundi *Lates calcarifer*, however, prey availability should be included in the model. When identifying whether a site was appropriate for the release of juvenile *A. japonicas*, Taylor *et al.* (2006) used the abundance of prey items as one of the determining factors to ensure that there was enough food available to support the density of the released population. This would not be applicable to all species, but is more important for higher trophic level species where abundance of prey items are more likely to be a limiting factor.

Abundance of predator species and fishing pressure

In our experiment the only predation considered was that by teleosts (although scyphozoans were considered in the preliminary analyses), however, predation by other animals should also be considered for different species. For example, in a release program for Murray Cod, *Maccullochella peelii*, predation from piscivorous birds had a significant effect on post-release survival (Hutchinson *et al.*, 2012). Therefore, predation by birds should be incorporated into the model alongside other predators when designing a release strategy for *M. peelii*. This applies for all species that may have predators other than teleosts.

The level of fishing effort on released populations is another source of mortality that may affect the success of a release program, and could potentially be included as a factor. For example, if the selection of a release site was on a larger scale such as that between lakes, then the effect of fishing pressure may differ based on the accessibility of that lake. If the aim of a release program were to rebuild stocks for conservation, then the level of fishing pressure would be an important factor for selecting which lake to release in. Changes in management, such as reductions in fishing effort or introducing seasonal closures to fishing, may also be beneficial to the success of releases for restocking purposes.

3.4.4. Visualisation of the SMART results

Once the SMART has been refined based on the improvements suggested above, the output should be presented in a way that is simple and easy to interpret for researchers/aquaculturists, managers, recreational fishers and the broader community. A visual output of the SMART scores for each site, such as that shown in Figure 3.29, is likely to facilitate discussions about the selection of the optimal release site(s). This example was created for January 2014, the time period assessed as the best for release across the three years of available data. At this time, the best release site based on the overall SMART Score was Deep Water Point (80), with several other sites having scores of ≥ 70 (*i.e.* Coode St, Rossmoyne,

Dalkeith and Matilda Bay, Fig 3.29). These figures provide valuable background for informed decision making on optimising the release strategies through the selection of the best release site for cultured *M. dalli* when a batch is ready for release. All the data required to populate the model, with the exception of sediment composition (which is readily available from Bennett, 2014), comes from a faunal monitoring regime. The entire sampling regime to provide the prerequisite data takes three evenings with researchers spending about ~20 minutes at each site. As all data is collected in the field, there is no laboratory processing of samples, and thus the SMART calculation could be calculated immediately to provide fast advice on an optimal release site to stakeholders at any time.

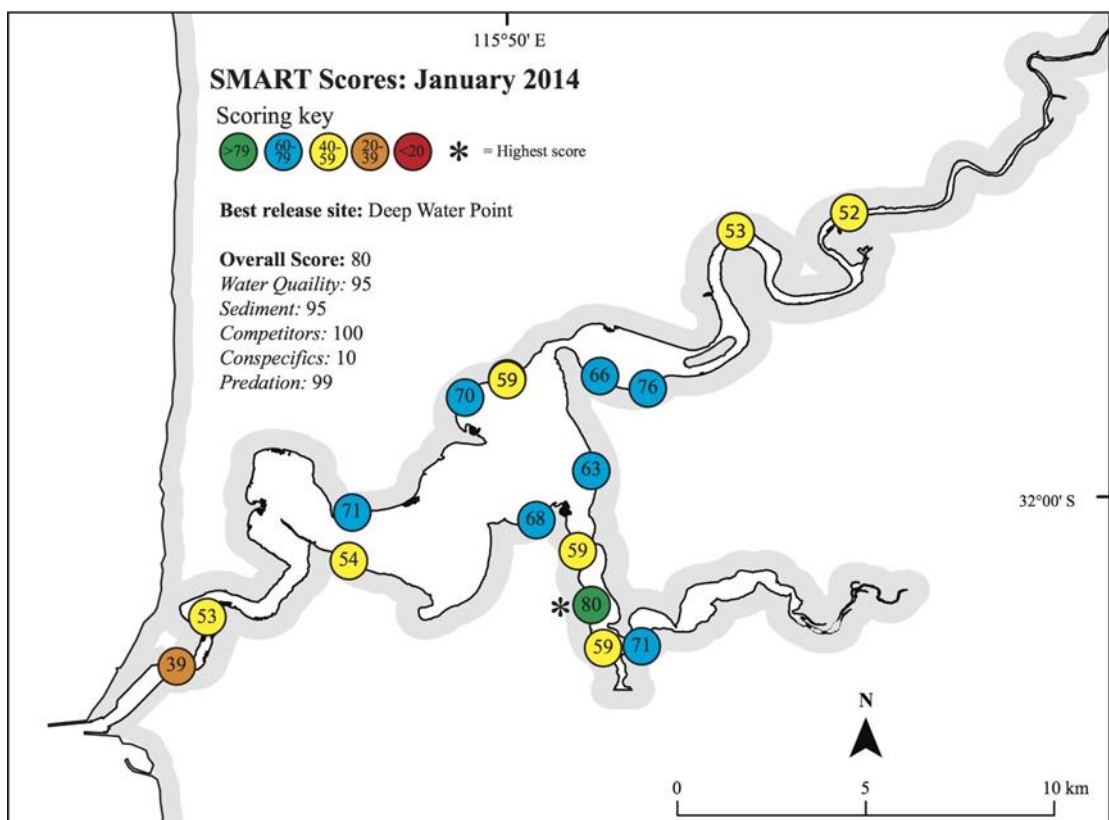


Fig. 3.29: Map showing the overall SMART scores for each of the 16 nearshore sites in the Swan-Canning Estuary for a night time release in January 2014.

Chapter 4: General conclusions and recommendations

4.1. Conclusions

The primary aim of this Thesis was to develop a quantitative tool to assist in the selection of optimal release sites and times for hatchery-reared *Metapenaeus dalli* in the Swan-Canning Estuary to support a restocking program. In Chapter 2, a range of factors were identified that should be considered when selecting appropriate release sites and times for a target species, including water quality (*e.g.* water temperature and salinity), availability of preferred habitat (in the case of *M. dalli* sediment composition), abundance of prey items and the presence of competitors, conspecifics and predatory species. Chapter 3 collated data on those factors identified as important to release strategies for *M. dalli*, along with further literature review and stakeholder engagement to evaluate the suitability of 16 potential release sites and five potential months for the release of hatchery-reared *M. dalli*, noting that it may not always be possible to select the timing of release. A methodology for synthesizing and quantitatively evaluating these factors, termed the Survival-Maximisation-At-Release-Tool (SMART), was developed in this component of the Thesis.

The SMART is, to the best of my knowledge, the first tool to quantitatively evaluate all data pertaining to the selection of an optimal release site and time. Through an extensive literature search, only a single study was found that employed a quantitative approach to facilitate release site selection, however, it used data only on habitat composition. Within the SMART, a comprehensive suite of biotic and abiotic variables were assessed at each site and time for their potential impact on post-release survival of *M. dalli* and standardised onto a common scale of 0-100. For example, the effect of salinity on the survival of prawns (Crisp *et al.*, 2017) was used to convert salinity recorded at the time of sampling into a percentage survival of *M. dalli* based on salinity at that site at the

time of sampling. Where needed, scores were inverted (*e.g.* as for predators, so that a large abundance of predators had lower scores) so that a score of 100 was always optimal for the survival of released *M. dalli*. These scores were then averaged to record an overall SMART score for each potential site and time, with the highest score identifying the optimal site or time.

Region was found to exert the greatest influence on the survival of hatchery-reared *M. dalli*, with regions in the middle of the estuary (*i.e.* Lower Canning Estuary and Perth Waters) recording the highest scores. The next most influential factor was years, followed by months. The variables used in the calculation of SMART that varied the most among regions and/or over time (months and year) were salinity, sediment composition and teleost predation. The optimal site and time of the year and day for release was during the night at Deep Water Point in the Lower Canning in January 2014. This site, at the time, had good water quality (*i.e.* a water temperature of ~ 26 °C and salinity ~ 36) and sediment composition (*i.e.* relatively low amounts of particulate organic matter and large contributions of fine sand particles) the complete absence of any competitor (*P. laticulatus*), as well as relatively high scores for conspecific *M. dalli* including those that were gravid and low abundances of teleost predators.

The result from this initial run of the SMART supports the view that an objective, quantitative approach for selecting release sites and times will facilitate the development of informed release strategies that help maximise the success of aquaculture-based enhancements. The tool showed significant differences in scores both spatially and temporally within the Swan-Canning Estuary. While this first iteration of the SMART shows promise, further modifications are likely to improve its reliability.

4.2. Future considerations

A number of improvements to the SMART are likely to enhance its ability to select release sites and times and the reliability of the results. These modifications include:

1. Further exploration into the effect of each identified variable on the survival of hatchery-reared *M. dalli* to ensure that the variables score is capturing the extent of these effects accurately. For example, while the model used estimates of survival in different water temperatures and salinity, these were based on the most sensitive larval stages not post-larval as will likely be released.
2. Improved weighting of variables within and between factors and factors to take into account potential differences in the scale of the effects. For instance, adjusting variables weights within a factor so that salinity has a greater effect on the water quality score than temperature and/or weighting one factor (*e.g.* predation) more than another because of its significance immediately post-release.
3. A more robust sampling regime, similar to that used to generate data for the night, for the collection of daytime data. This would allow a more accurate assessment of the optimal day release sites and also facilitate a more precise comparison of the advantages of release in the day *vs* night or *vice versa* and whether this changes spatially and temporally.
4. Evaluating the optimal release size for *M. dalli* to incorporate in the model, *e.g.* is survival likely to be significantly enhanced by releasing small juvenile prawns instead of 3 mm long post-larvae.

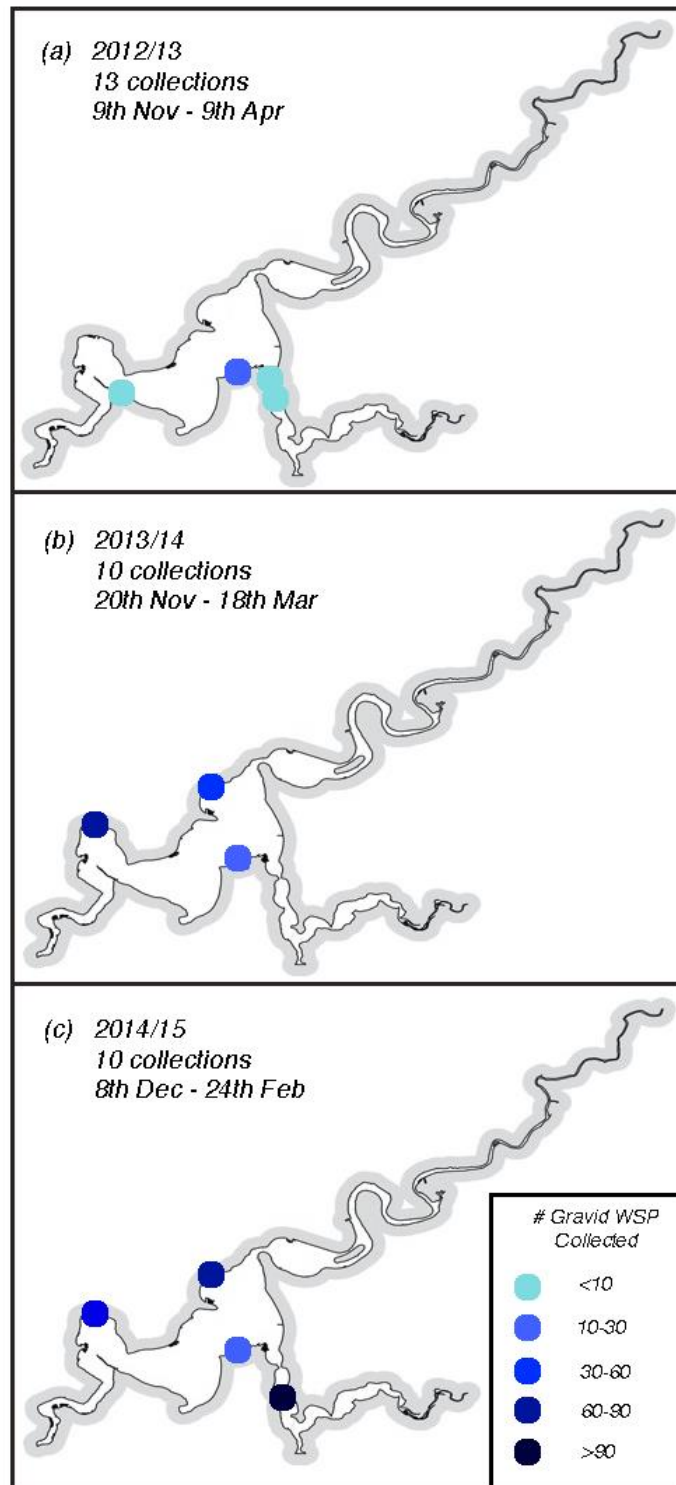
Appendices

Appendix 1: SMART Scores for each month/year at each site during night-time. Colouring in each cell represents the SMART score ● <40, ● 41-49, ● 51-60, ● 60-69,

● 70-79, ● 80+.

Sites	Months/Years														
	Nov-13	Dec-13	Jan-14	Feb-14	Mar-14	Nov-14	Dec-14	Jan-15	Feb-15	Mar-15	Nov-15	Dec-15	Jan-16	Feb-16	Mar-16
Stirling Bridge	35	40	38	44	42	53	56	58	55	50	52	50	49	34	52
Leeuwin Barracks	36	41	51	42	60	55	57	61	59	48	61	55	54	40	51
Point Walter	56	53	52	55	53	58	68	72	53	51	69	68	55	43	51
Attadale	50	59	66	58	60	58	56	66	64	63	58	58	49	48	52
Como	52	52	61	69	57	59	59	58	66	55	66	54	57	52	52
Dalkeith	66	68	69	76	73	60	74	71	65	75	66	76	63	58	59
Matilda Bay	52	53	68	68	60	63	70	70	66	53	68	65	64	68	52
Kings Park	55	64	57	62	60	58	61	61	56	58	58	64	76	65	58
Coode st	65	67	74	62	55	71	75	68	64	64	75	74	64	66	61
South perth	71	70	64	76	75	76	78	63	73	66	75	69	66	61	70
Canning Bridge	73	59	58	70	64	60	71	63	63	65	80	74	75	56	64
Deep Water Point	75	76	80	76	61	76	75	72	72	74	70	76	71	66	58
Freeway	72	76	57	73	63	71	73	65	57	58	68	72	62	61	61
Rossmoyne	70	68	69	57	60	70	73	67	63	59	75	57	65	63	58
Maylands	59	60	51	66	57	62	59	67	68	66	68	61	60	52	67
Garratt rd Bridge	58	57	50	57	61	56	57	55	63	59	50	47	49	52	57

Appendix 2: Map of the Swan-Canning Estuary indicating areas where broodstock collection took place in each of the three years of the pilot study. Coloured circles indicate the approximate number of gravid female *M. dalli* collected. Data taken from Jenkins *et al.* (2015)

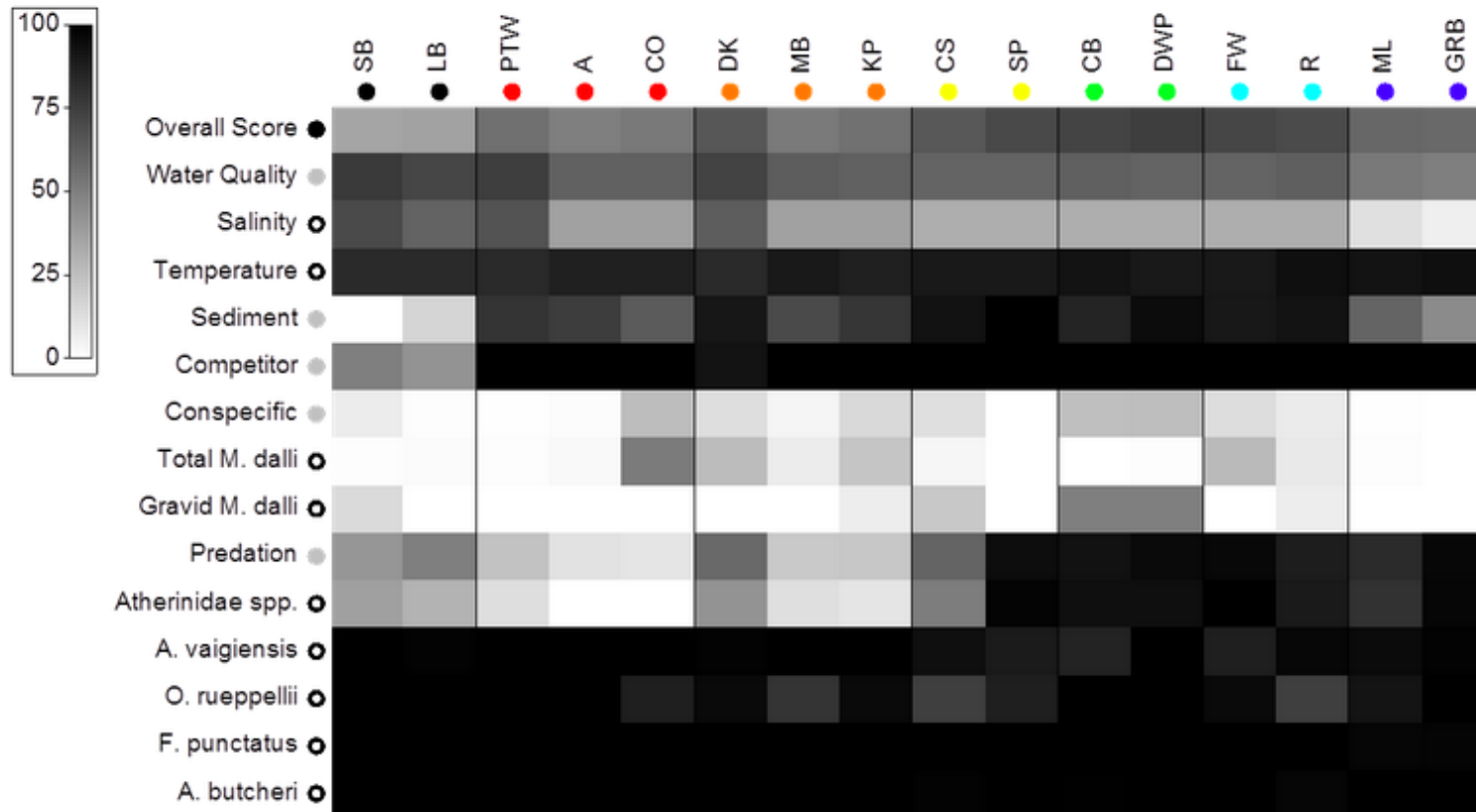


Appendix 3: List of all fish and scyphozoan species recorded in the daytime (day) and nighttime (night) sampling regimes and whether included in the initial (model) and final (SMART) calculation of SMART.

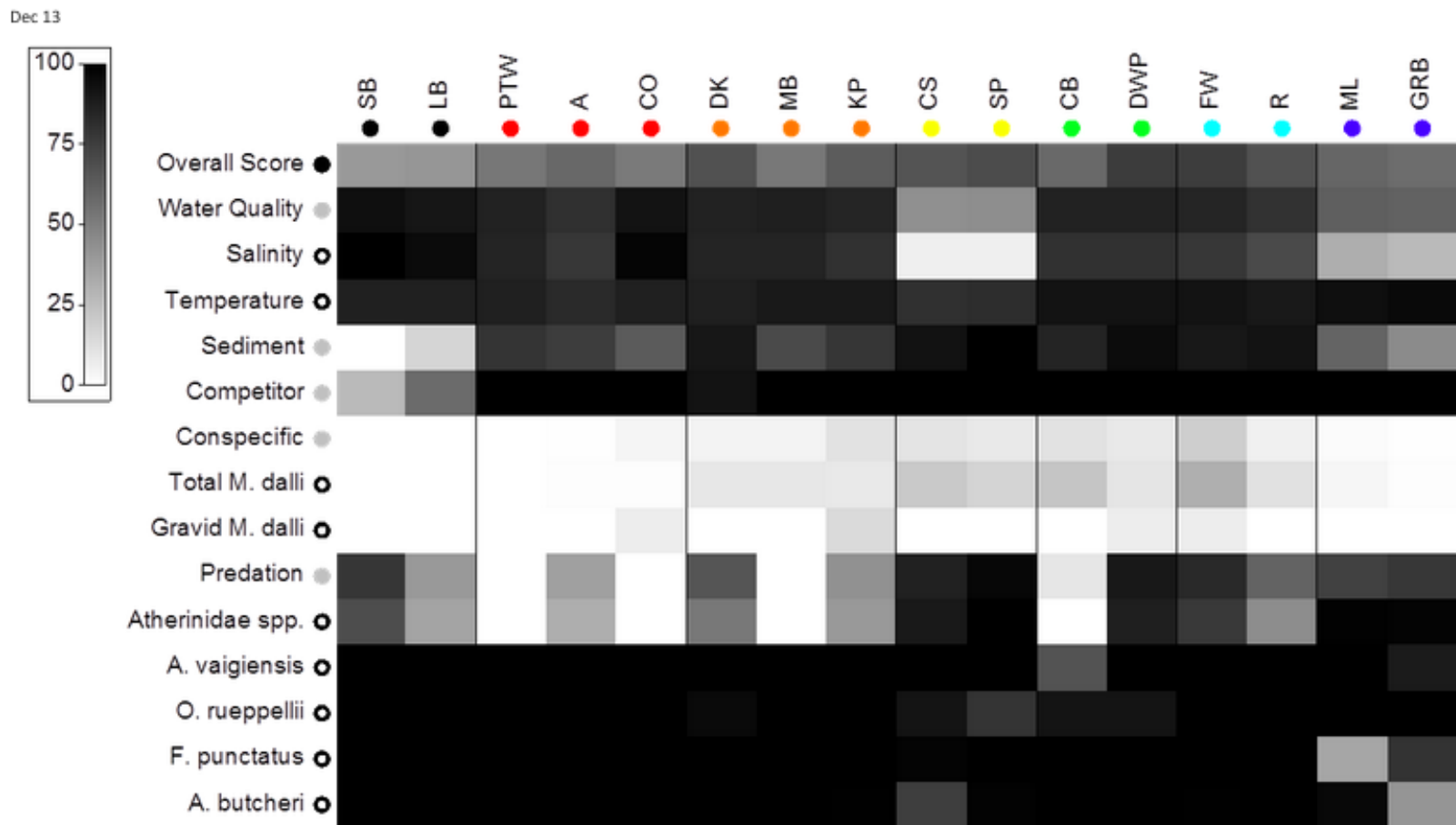
Species	Common Name	Day	Night	Prelim Analysis	SMART
Teleost species					
<i>Arenigobius bifrenatus</i>	Bridled Goby				
<i>Acanthaluteres brownii</i>	Spinytail Leatherjacket				
<i>Acanthopagrus butcheri</i>	Black Bream				
<i>Amniataba caudavittata</i>	Yellowtail Trumpeter				
<i>Atherinosoma elongata</i>	Elongate Hardyhead				
<i>Aldrichetta forsteri</i>	Yellow-eye Mullet				
<i>Arripis georgianus</i>	Australian Herring				
<i>Ammotretis rostratus</i>	Longsnout Flounder				
<i>Acanthaluteres spilomelanurus</i>	Bridled Leatherjacket				
<i>Afurcagobius suppositus</i>	Long-headed Goby				
<i>Atherinomorus vaigiensis</i>	Common Hardyhead				
<i>Cnidogobius macrocephalus</i>	Estuary Cobbler				
<i>Craterocephalus mugiloides</i>	Spotted Hardyhead				
<i>Epinephelides armatus</i>	Black-arse Cod				
<i>Engraulis australis</i>	Australian Anchovy				
<i>Elops machnata</i>	Tenpounder				
<i>Fistularia commersonii</i>	Bluespotted cornetfish				
<i>Favonigobius lateralis</i>	Southern Longfin Goby				
<i>Favonigobius punctatus</i>	Yellowspotted Sandgoby				
<i>Geophagus brasiliensis</i>	Pearl Cichlid				
<i>Gambusia holbrooki</i>	Eastern Gambusia				
<i>Gerres subfasciatus</i>	Common Silverbiddy				
<i>Hippocampus angustus</i>	Western Spiny Seahorse				
<i>Hyporhamphus melanochir</i>	Southern Sea Garfish				
<i>Haletta semifasciata</i>	Blue-weed Whiting				
<i>Hydrocynus vittatus</i>	African Tigerfish				
<i>Leptatherina presbyteroides</i>	Swan-River Hardyhead				
<i>Leptatherina wallacei</i>	Western Hardyhead				
<i>Mugil cephalus</i>	Flathead Grey Mullet				
<i>Neoodax balteatus</i>	Little Weed Whiting				
<i>Nematalosa vlaminghi</i>	Western Australian Gizzard Shad				
<i>Ostorhinchus ruepellii</i>	Western Gobbleguts				
<i>Pugnaso curtirostris</i>	Pugnose Pipefish				
<i>Pseudocaranx dentex</i>	White Trevally				
<i>Platycephalus endrachtensis</i>	Bar-tailed Flathead				
<i>Parapercis haackei</i>	Wavy Grubfish				
<i>Pelsartia humeralis</i>	Sea Trumpeter				
<i>Pseudorhombus jenynsii</i>	Small-Toothed Flounder				
<i>Platycephalus laevigatus</i>	Rock Flathead				
<i>Pelates octolineatus</i>	Western Striped Grunter				
<i>Pseudogobius olorum</i>	Swan River Goby				
<i>Rhabdosargus sarba</i>	Goldlined Seabream				
<i>Stigmatophora argus</i>	Spotted Pipefish				
<i>Sillago bassensis</i>	Western School Whiting				
<i>Sillago burrus</i>	Western Trumpeter Whiting				
<i>Sillago maculata</i>	Trumpeter Whiting				
<i>Stigmatophora nigra</i>	Widebody Pipefish				
<i>Spratelloides robustus</i>	Blue Sprat				
<i>Sillago schomburgkii</i>	Yellowfinned Whiting				
<i>Torquigener pleurogramma</i>	Weeping Toadfish				
<i>Urocampus carinirostris</i>	Hairy Pipefish				
Scyphozoan species					
<i>Phyllorhiza punctata</i>	Brown Jelly				
<i>Aurelia aurata</i>	Moon Jelly				

Appendix 4: Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in November 2013 during night-time. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.

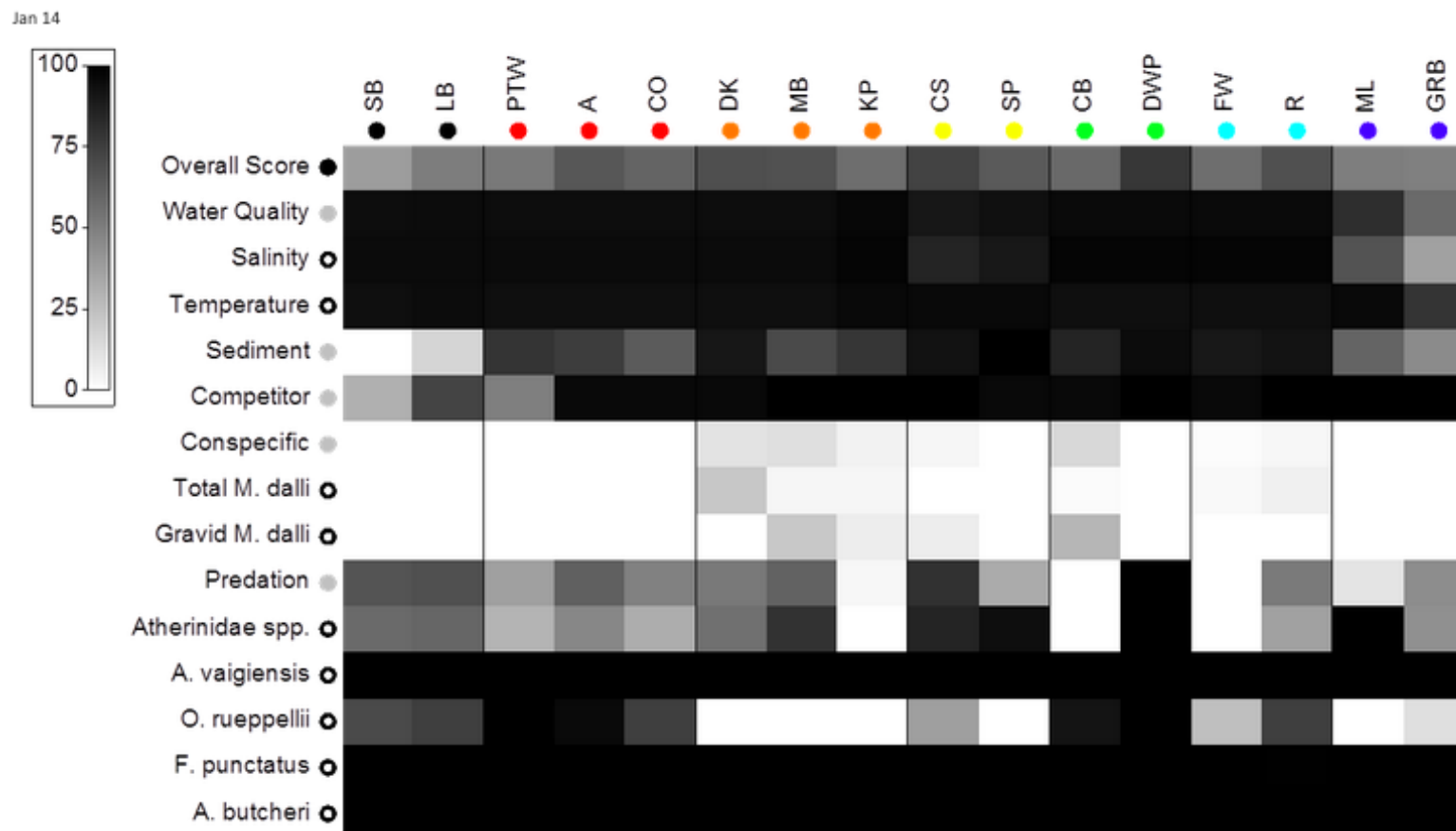
Nov 13



Appendix 5: Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in December 2013 during night-time. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.

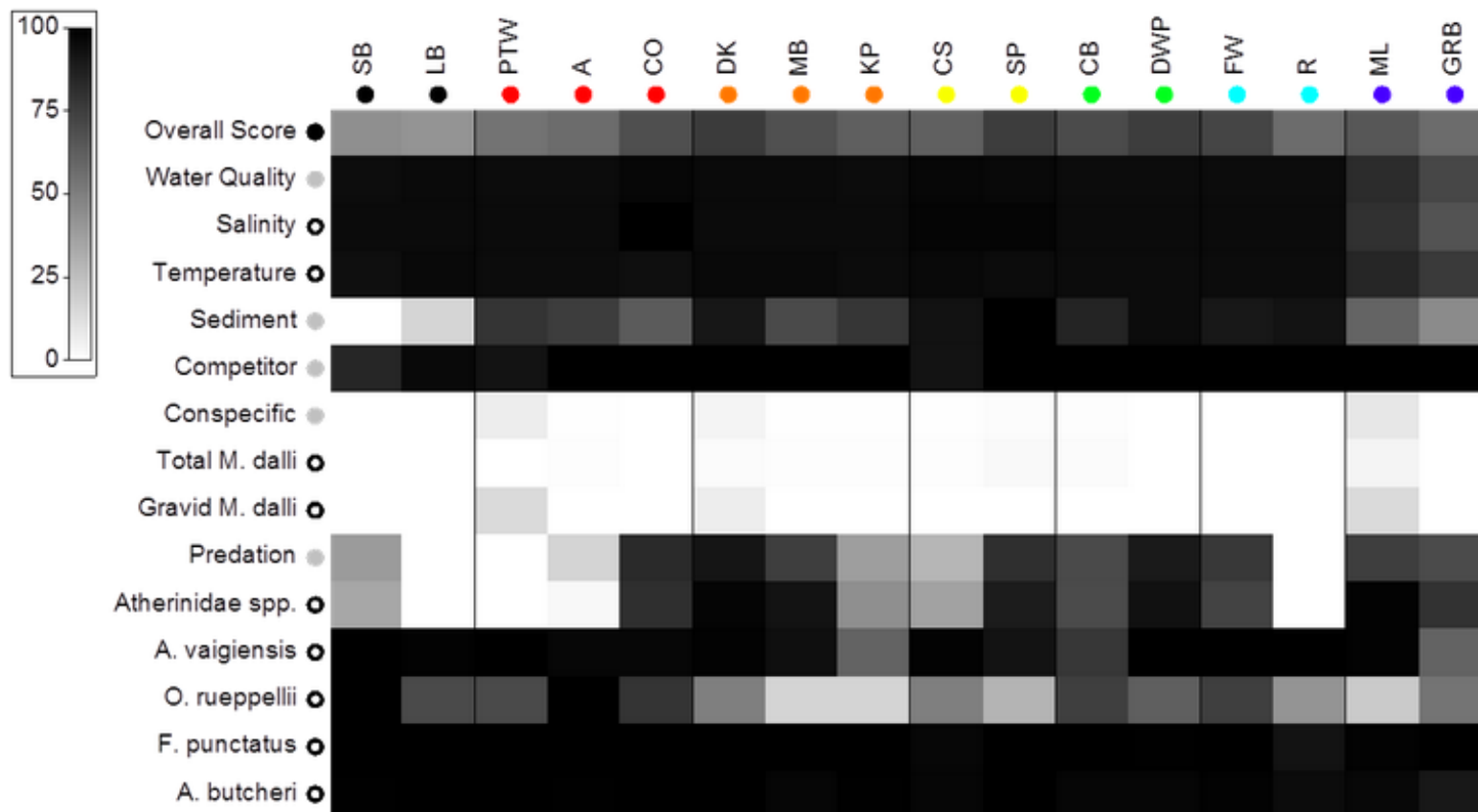


Appendix 6: Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in January 2014 during night-time. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.

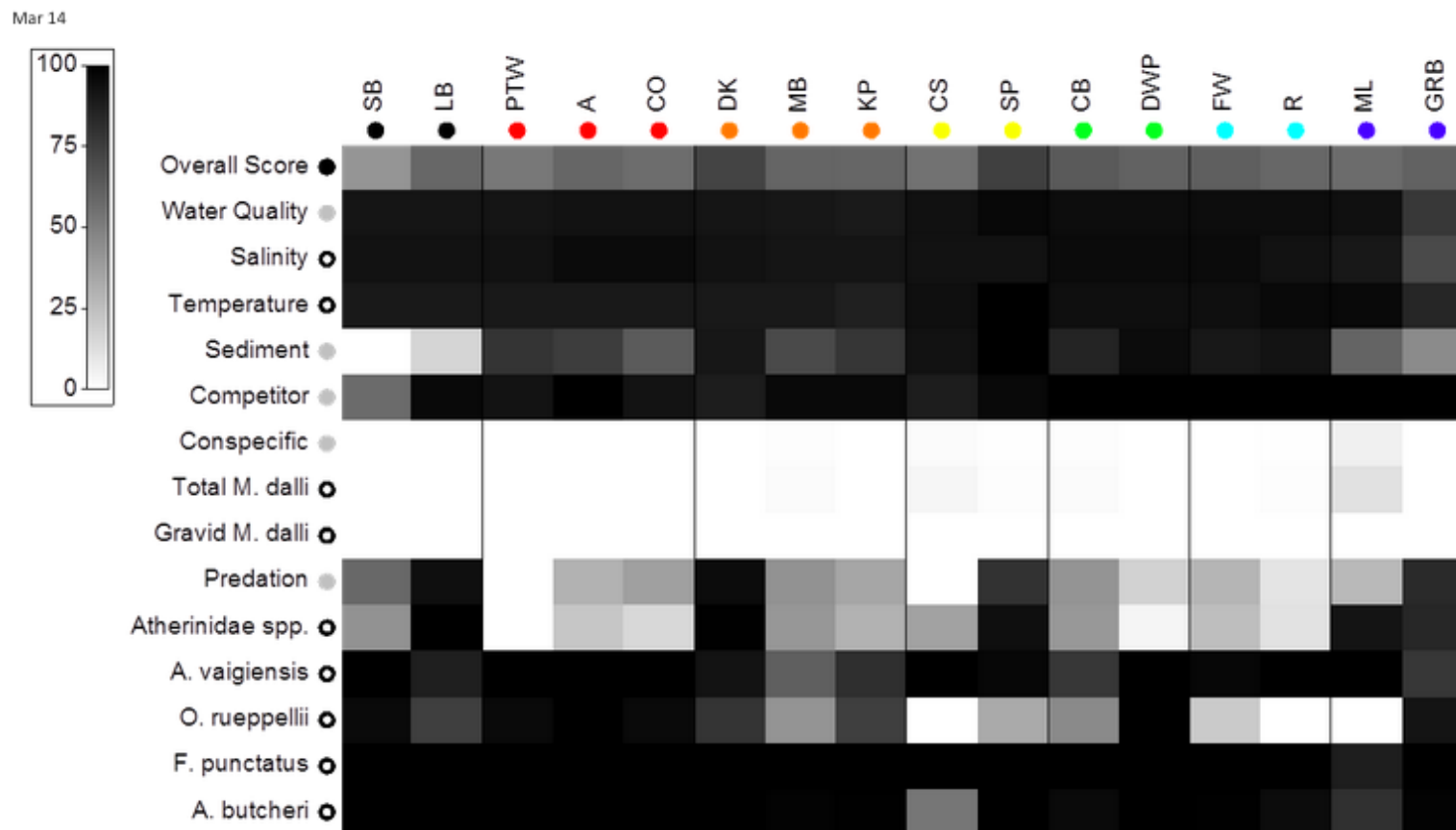


Appendix 7: Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in February 2014 during night-time. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.

Feb 14

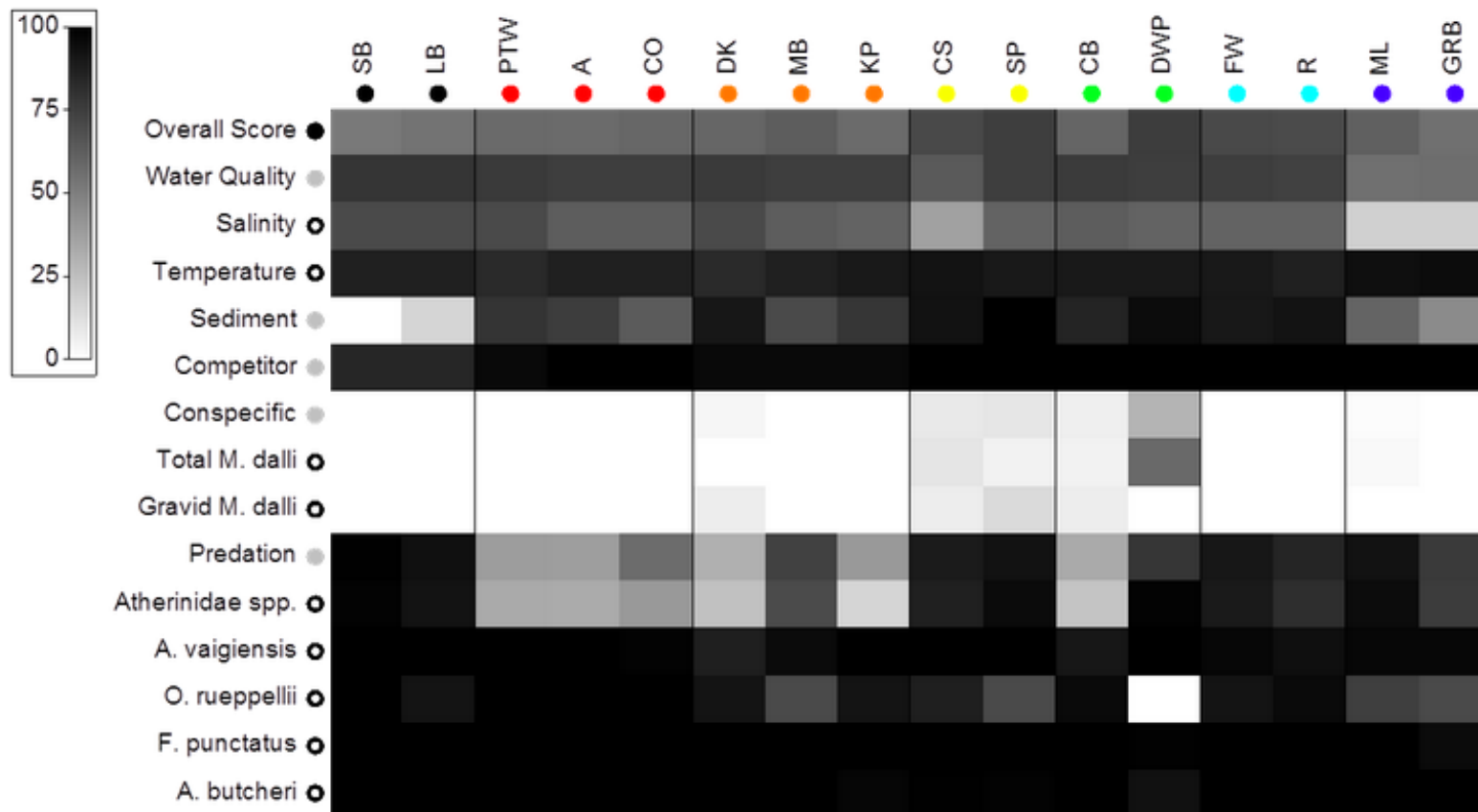


Appendix 8: Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in March 2014 during night-time. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.

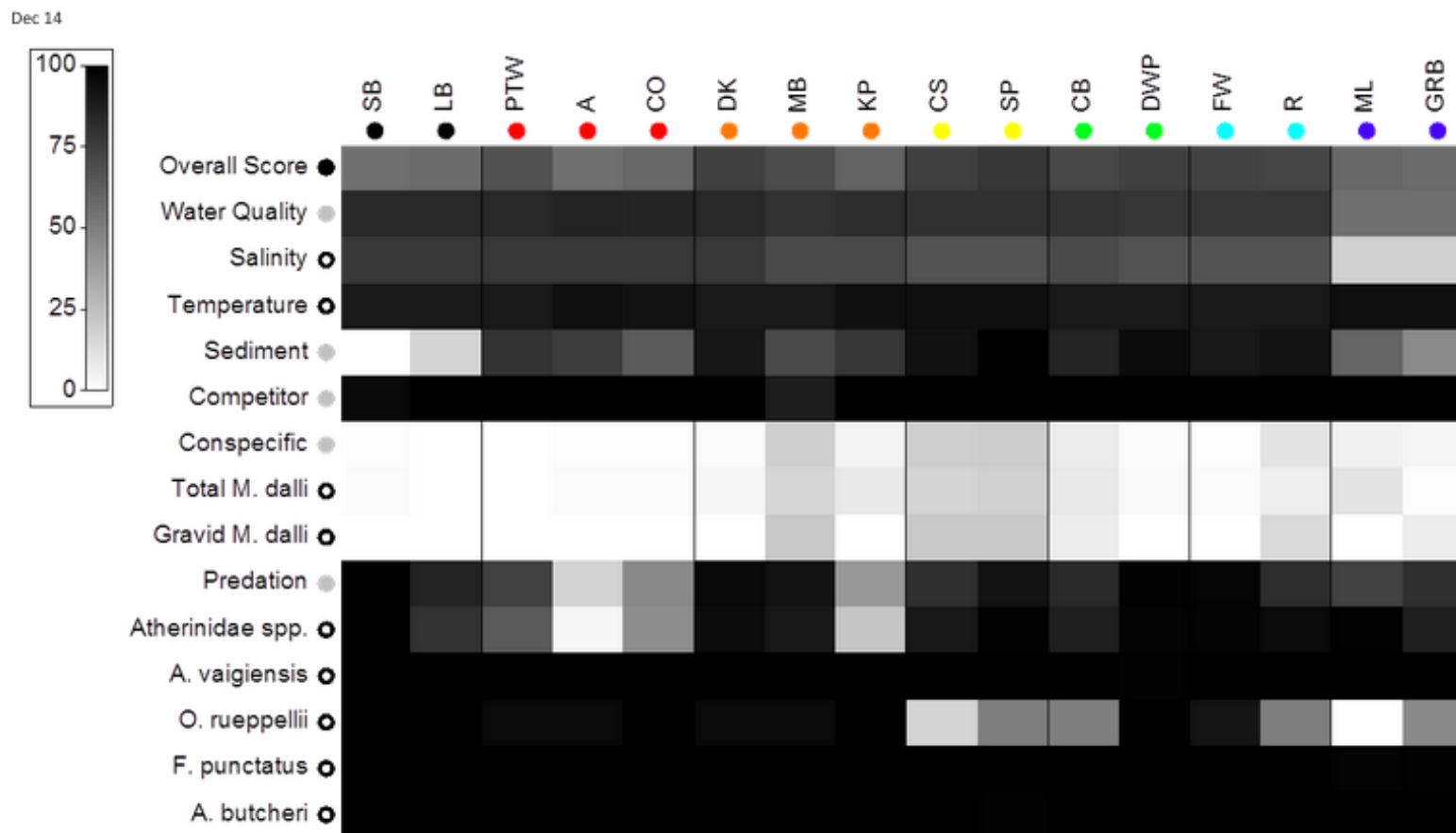


Appendix 9: Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in November 2014 during night-time. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.

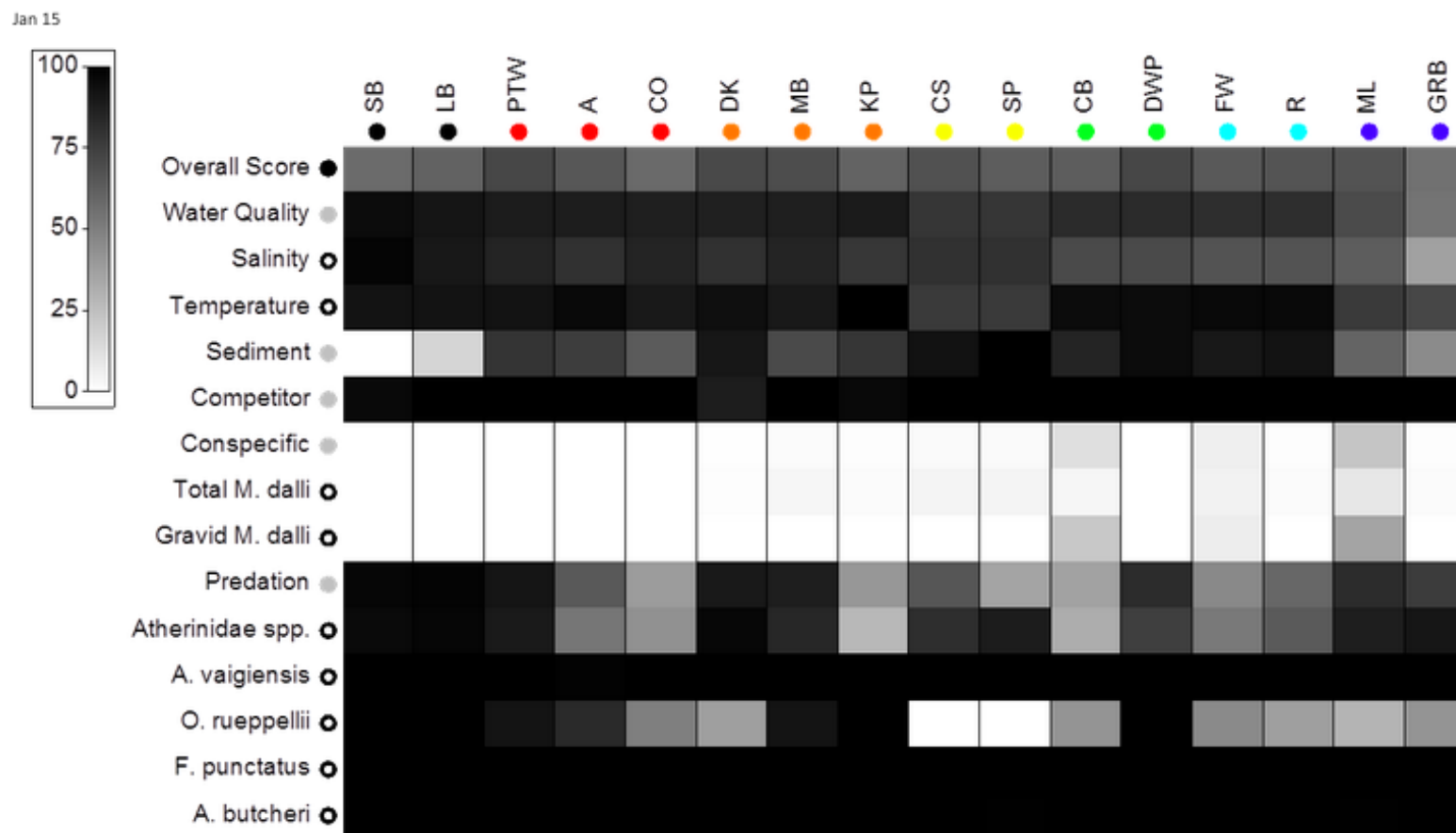
Nov 14



Appendix 10: Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in December 2014 during night-time. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.

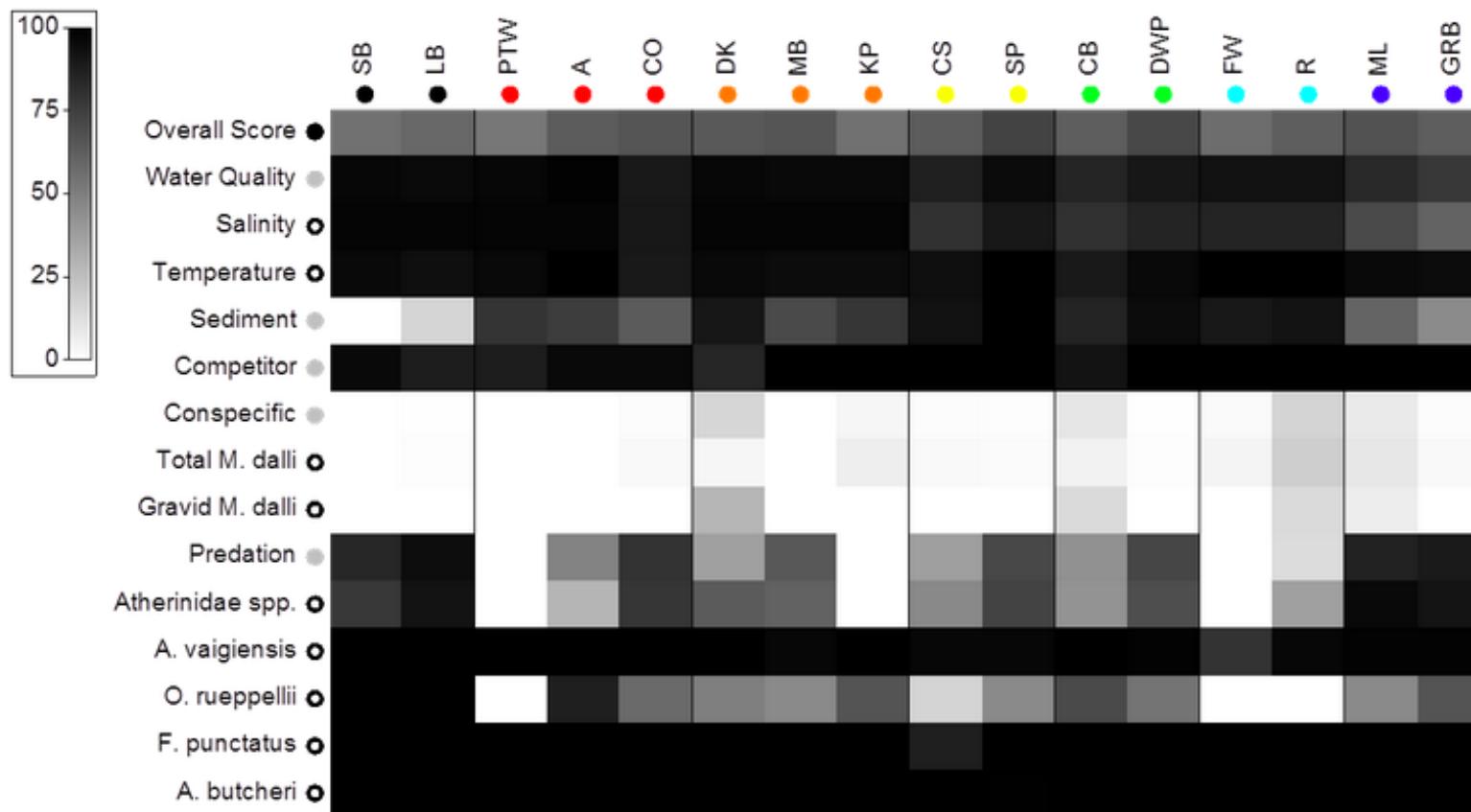


Appendix 11: Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in January 2015 during night-time. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.

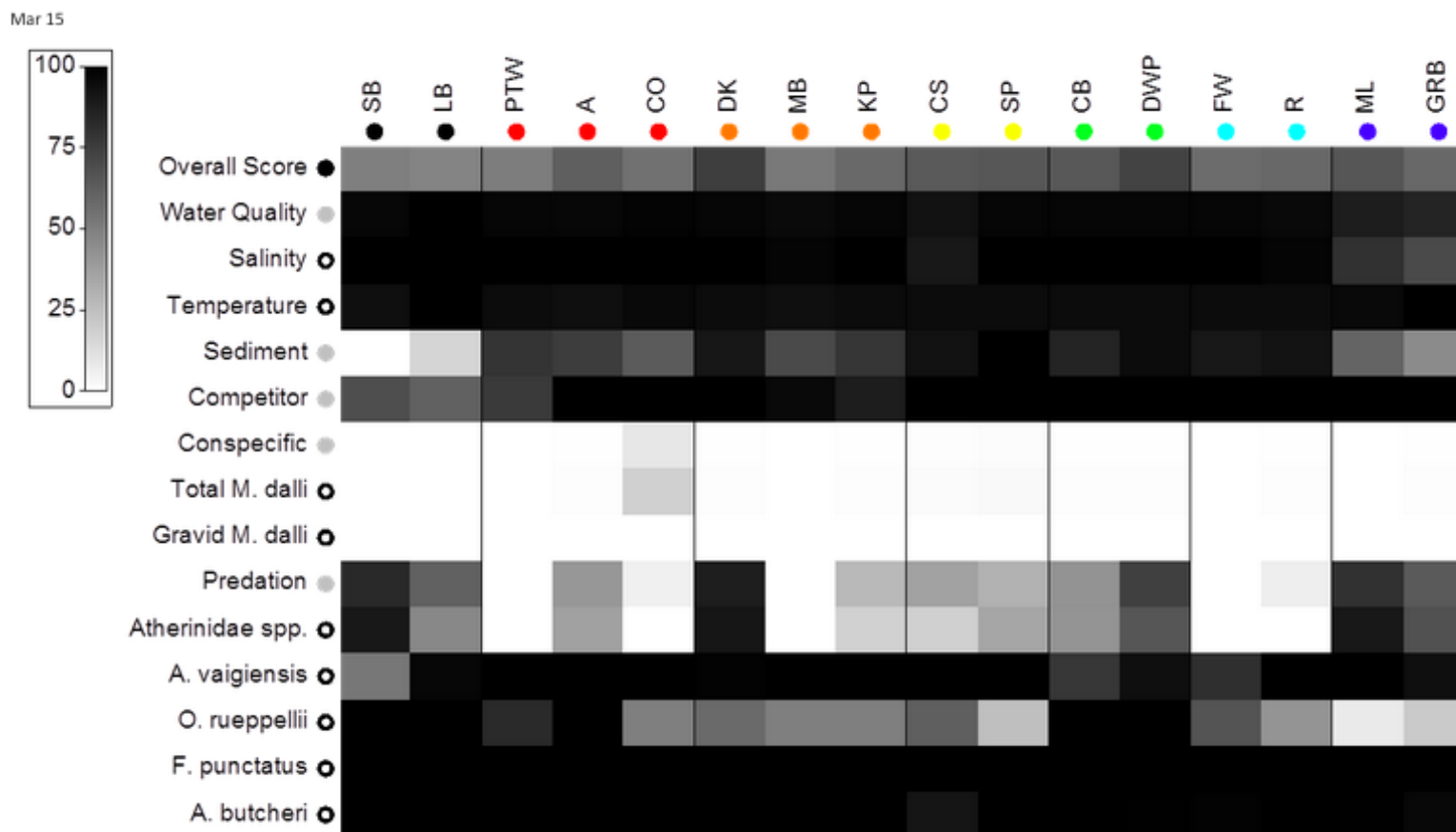


Appendix 12: Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in February 2015 during night-time. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.

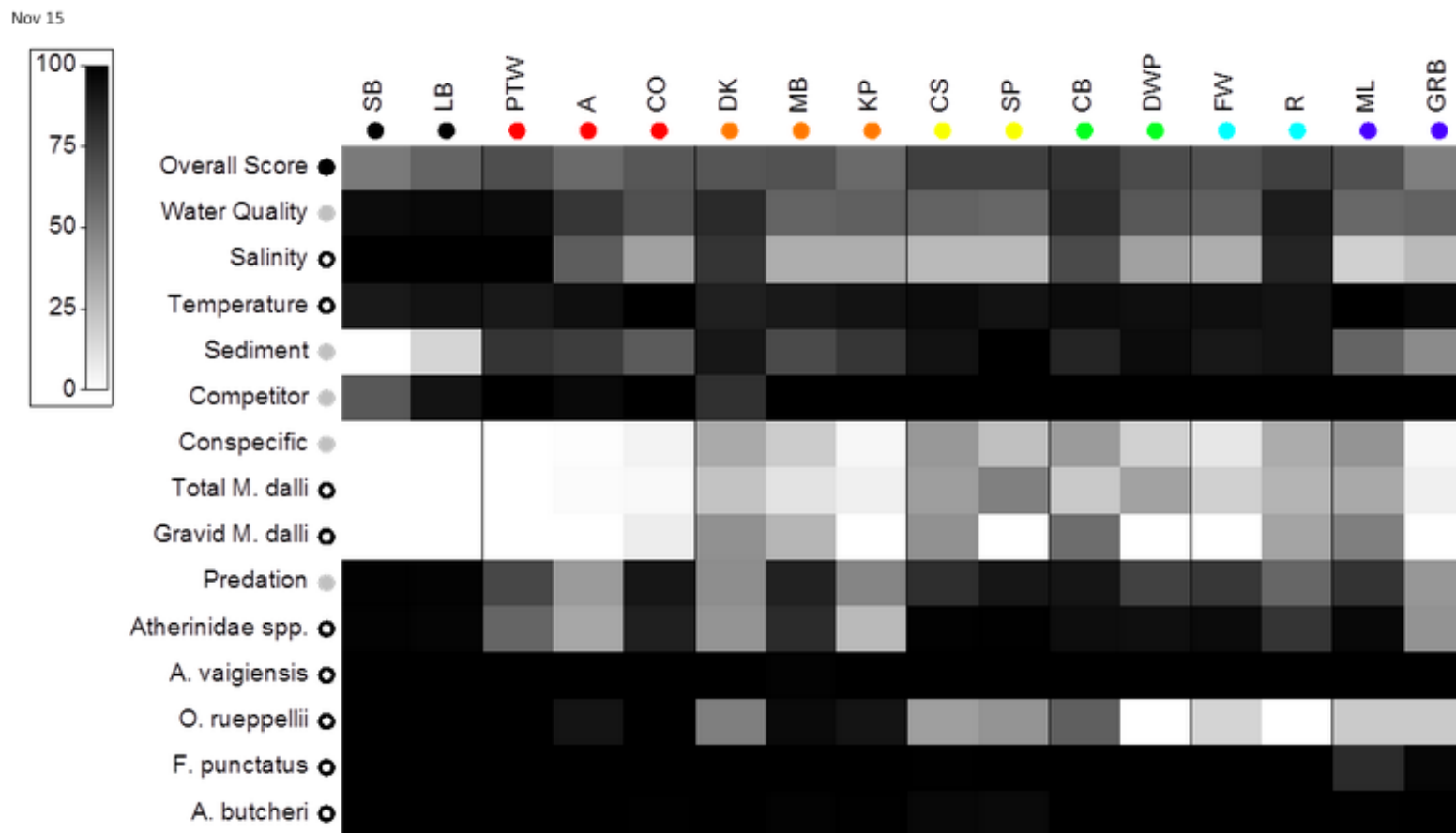
Feb 15



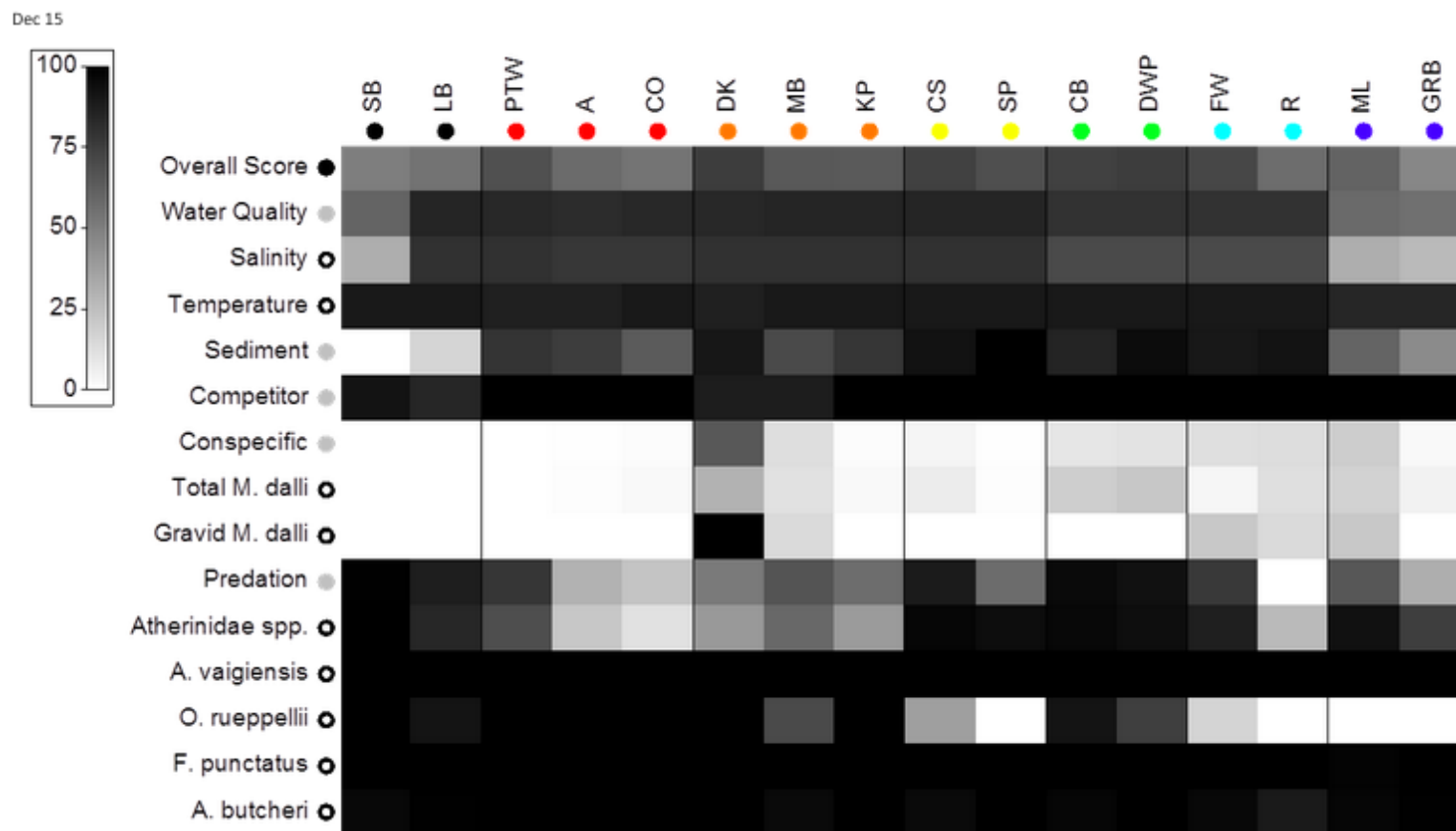
Appendix 13: Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in March 2015 during night-time. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.



Appendix 14: Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in November 2015 during night-time. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.

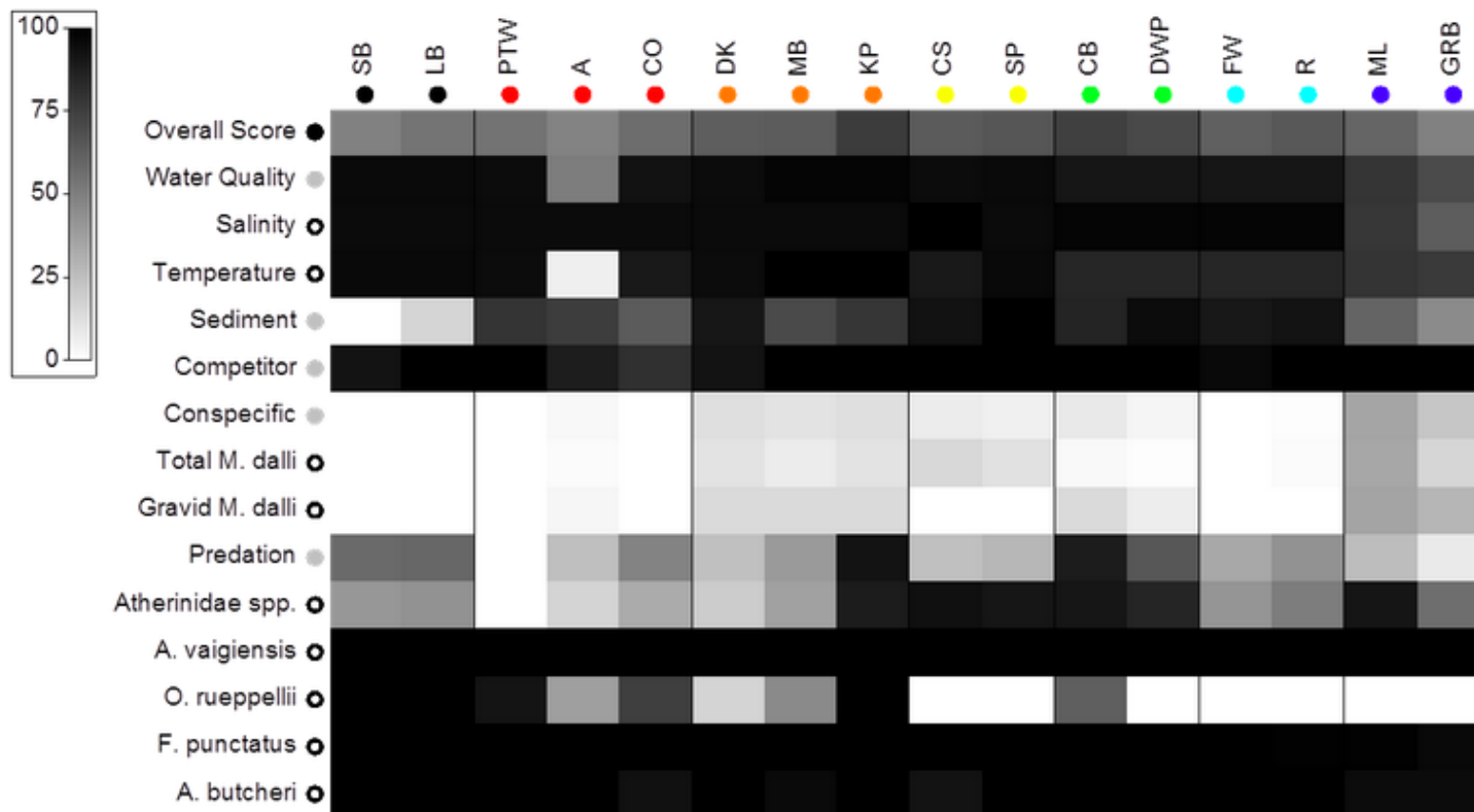


Appendix 15: Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in December 2015 during night-time. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.



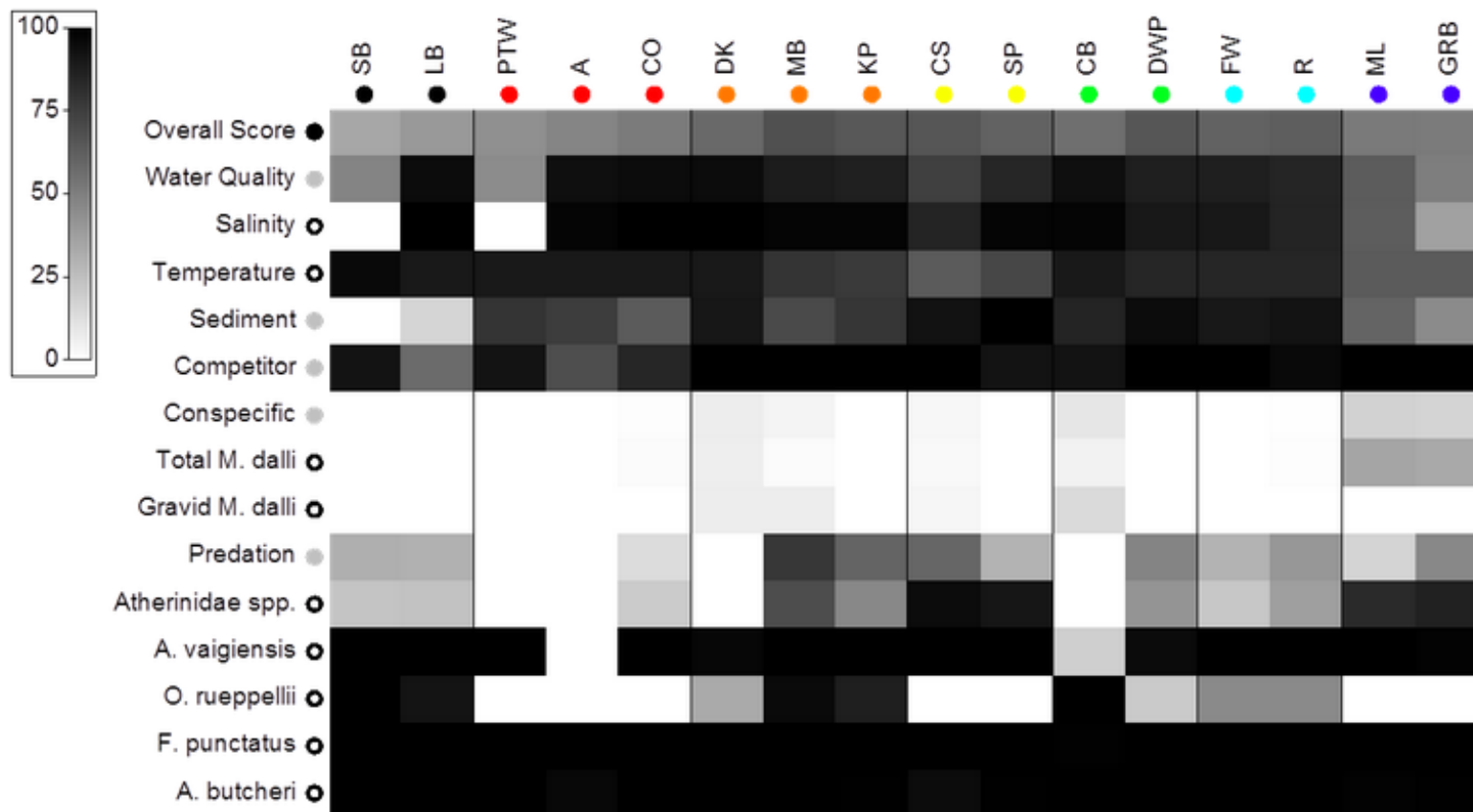
Appendix 16: Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in January 2016 during night-time. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.

Jan 16

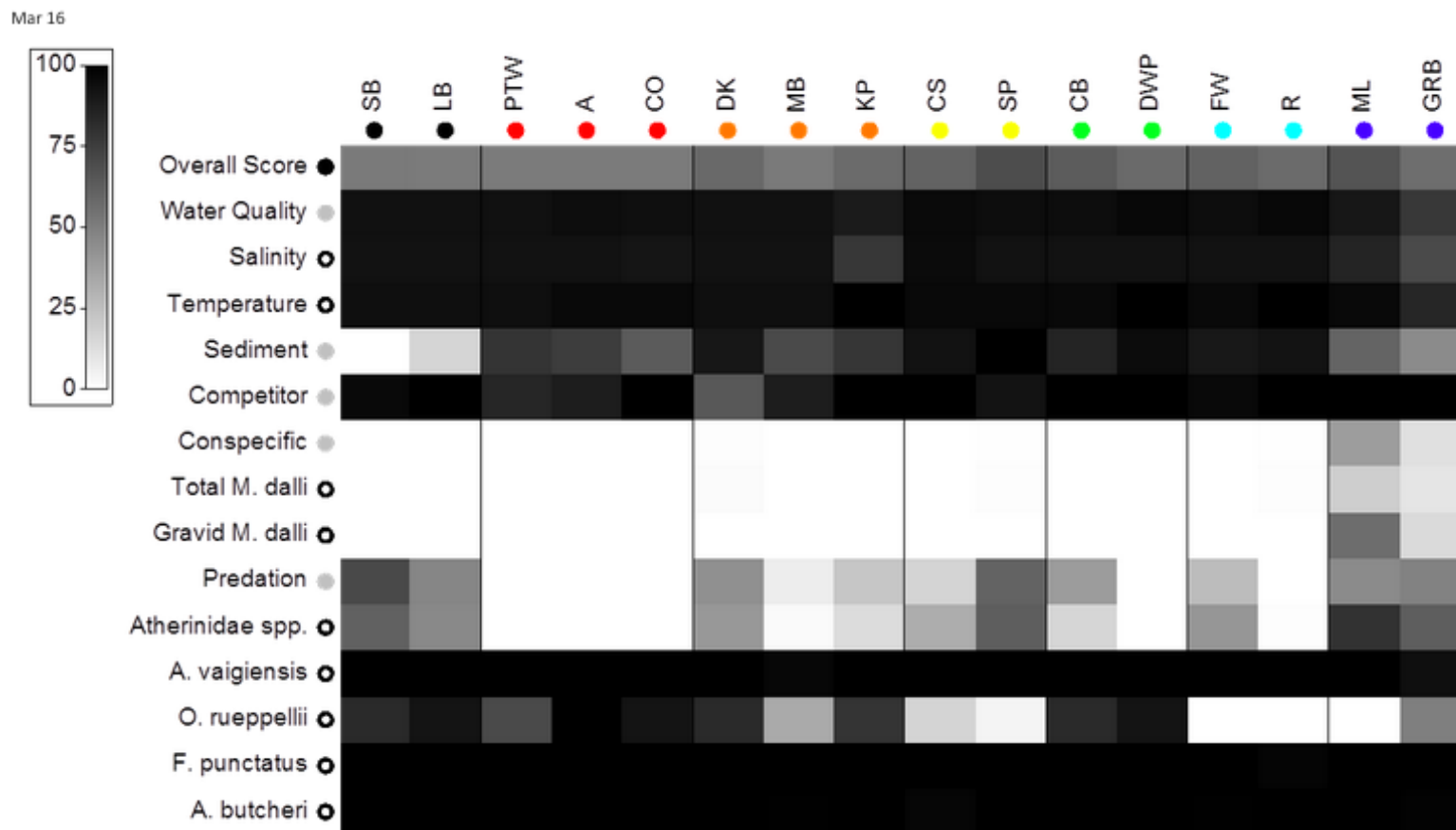


Appendix 17: Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in February 2016 during night-time. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.

Feb 16



Appendix 18: Shade plot, constructed using the raw overall SMART score (●) and the raw scores for each factor (●) and variable (○) for each of the 16 sites in the nearshore waters of the Swan-Canning Estuary in March 2016 during night-time. The greyscale from white to black denotes increasing SMART scores and thus a better release site for hatchery-reared *M. dalli*. Coloured circle denotes the regions to which a site belongs (see Fig. 3.1); Entrance Channel (●), South Melville Water (●), North Melville Water (●), Perth Water (●), Lower Canning Estuary (●), Upper Canning Estuary (●) and the Middle Swan Estuary (●) and the text code the site, *i.e.* SB = Stirling Bridge, LB = Leeuwin Barracks, PTW = Pt Walter, A = Applecross, CO = Como, DK = Dalkeith, MB = Matilda Bay, KP = Kings Park, CS = Coode St, SP = South Perth, CB = Canning Bridge, DWP = Deep Water Point, FW = Freeway, R = Rossmoyne, ML = Maylands and GRB = Garratt Rd Bridge.



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