



Assessment of the potential impacts on threatened fish from the construction, filling and operation of the Enlarged Cotter Dam: Phase 1 Basline

2010 – 2012

Mark Lintermans, Ben Broadhurst And Rhian Clear

Institute for Applied Ecology

Ecological Solutions for a Healthy Environment

University of Canberra,
ACT 2601, AUSTRALIA

Phone: (02) 6201 2795
Fax: (02) 6201 5305
Web: www.appliedecology.edu.au
Email: enquiry@iae.canberra.edu.au

UNIVERSITY OF CANBERRA
AUSTRALIA'S CAPITAL UNIVERSITY

Assessment of the potential impacts on threatened fish from the construction, filling and operation of the Enlarged Cotter Dam Phase 1 (2010-2012): Final Report

Report prepared for: ACTEW Corporation

Authors: Mark Lintermans, Ben Broadhurst, Rhian Clear

Produced by:
Institute for Applied Ecology
University of Canberra, ACT 2601
Telephone: (02) 6201 2795 Facsimile: (02) 6201 5651
Website: http://www.canberra.edu.au/centres/iae/index.php
ABN: 81 633 873 422

Inquiries regarding this document should be addressed to:
Mark Lintermans
Phone: 02 6201 2853 Fax: 02 6201 5651
Email: Mark.Lintermans@canberra.edu.au

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Cover photo: Kissops Flat (a reference site) on the Murrumbidgee River: inset juvenile Macquarie perch (Photographs: Mark Lintermans).

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EXECUTIVE SUMMARY

The construction and operation of the Enlarged Cotter Dam (ECD) has been identified as having a range of potential impacts on fish populations in the ECD itself and the river upstream. The major concerns focused on the population of the nationally endangered Macquarie perch in Cotter Reservoir and the river upstream. After a risk assessment of these potential impacts, a series of management questions were constructed around potentially significant impacts to form the basis of an assessment program. The 12 management questions are;

Q1) Will there be significant changes in the abundance of Macquarie perch in the ECD (Young-of-Year, juveniles and adults)?

Q2) Will there be a significant change in annual recruitment in the Macquarie perch population in the ECD relative to a reference site (Kissops Flat)?

Q3) Will there be significant changes in the abundance, distribution and size composition of adult trout in the ECD?

Q4) What are the levels of predation on Macquarie perch larvae and juveniles by trout in the ECD and river upstream?

Q5) Will there be significant changes in the abundance, distribution and species composition of piscivorous birds in the ECD?

Q6) Will there be significant changes in the abundance and size composition of trout in the Cotter River upstream of the ECD?

Q7) Will there be a significant increase in the levels of predation on Two-spined blackfish by trout in the Cotter River upstream of the ECD?

Q8) Will there be significant changes in the abundance and distribution of the Macquarie perch population in the Cotter River above and below Vanitys Crossing?

Q9) Will macrophyte beds re-establish in the enlarged ECD?

Q10) Will translocated Macquarie perch populations survive the initial translocation procedure and reproduce?

Q11) Will Two-spined blackfish establish a reproducing population in the ECD and will they persist in the newly inundated section of the river?

Q12) Will there be significant changes in the abundance and distribution of Goldfish and Oriental weatherloach in the ECD?

Question 9 cannot be assessed until the enlarged Cotter Reservoir has reach Full Supply Level. Question 10 is being addressed under a separate project.

The assessment program was designed to be conducted in three broad phases; baseline (Before the ECD starts to fill), filling (As the ECD fills) and operation (Once the ECD has filled and began operation as a water supply reservoir).Where possible the assessment program employs a before after control impact (BACI) study design to provide a robust regime for detecting change associated with the ECD. The study design is also field integrated with many of the field survey activities addressing multiple management questions. The assessment program has now been in operation for three years collecting baseline data. Despite adverse climatic conditions in the final two years of the project, the field survey component has been completed with very few missing data points. High flow associated with heavy rains prevented some sampling events in 2011 and 2012.

Macquarie perch were monitored both in the Cotter Reservoir and the Cotter River between Cotter and Bendora reservoirs, and at a reference site on the upper Murrumbidgee River. Trout diet was analysed to determine the level of predation of young-of-year (YOY) Macquarie perch in the Cotter River. Adult Macquarie perch captures from the baseline data collection in Cotter Reservoir are relatively consistent across the three years of assessment in this project and that of previous work conducted in 2008-09. The only obvious inconsistency was two nights where no Macquarie perch were captured in 2012, when the water level in Cotter Reservoir was well above Full supply level (FSL). It is considered that the inundated vegetation around the perimeter of the reservoir resulted in sub-optimal deployment of gill nets and impeded capture of adult Macquarie perch. The current study recommends investigating alternative sampling techniques for adult Macquarie perch in Cotter Reservoir to counter this sampling problem during filling and early operation phase of the ECD. Recruitment of YOY Macquarie perch was detected at Cotter Reservoir and the reference site in all three years, though considerable inter-annual variation was evident at both sites. A large number of YOY Macquarie perch were captured in the reservoir in 2010 compared to 2011 and 2012 and historical catches. This indicates that the spawning and early recruitment from 2009 was extremely successful and that there is likely significant inter-annual variation in recruitment of Macquarie perch YOY in Cotter Reservoir.

Abundance of Macquarie perch as detected by fyke netting fluctuated between years at both the Cotter River sites and the reference site. The variation between years was largely driven by the presence of large numbers of age 1+ and 2+ individuals at a site (Vanitys crossing) in the Cotter River, and by the presence of YOY individuals at the reference site. Macquarie perch distribution in the Cotter River has not changed over the three years of assessment and is congruent with distribution data collected in 2007 and 2008. The current study recommends that a second riverine reference site be used to attempt to better understand inter-annual variations in the reference Macquarie perch population.

Heavy rains and resultant high river flows prevented the collection of trout diet samples in spring of 2010 and 2011. Data from 2012 did not detect any Macquarie perch in the diets of the 37 trout analysed. It is possible that there was predation of larval and juvenile Macquarie perch in 2012, but this was not detected in the visual analysis of dietary items conducted in the current study. Progress was made in developing a laboratory test to allow detection of Macquarie perch DNA from stomach samples, but further development is required before this test can be reliably deployed.

Two-spined blackfish were found for the first time in 30+ years to be present in Cotter Reservoir (in 2012). It is believed that the individuals captured may have been displaced by high flow and / or colonised the above-FSL backed up waters of the Cotter Reservoir during the 2011 floods. The colonisation of Cotter Reservoir by Two-spined blackfish appears to be a positive indicator for how this species will respond in the ECD. Three years of monitoring revealed low abundances of Two-spined blackfish at the riverine sampling site which will be inundated when the ECD fills (Bracks Hole). This is consistent with previous studies after the 2003 ACT bushfires. Two-spined blackfish population abundance elsewhere in the Cotter River appears relatively stable, with some site and inter-annual variability observed.

Incidences of trout predation on Two-spined blackfish were low, with only 5 confirmed records from 710 trout stomachs analysed (predominantly Rainbow trout but some Brown trout). All trout that were found to have consumed Two-spined blackfish were greater than 250 mm fork length. It is likely that this predation rate is an underestimate (as a result of fish remains being visually unidentifiable) but reflects a repeatable and indicative method to determine the level of predation.

The abundance and distribution of predators (piscivorous birds and trout spp.) of threatened native fish was assessed. Visual surveys revealed fluctuations in the abundance of piscivorous birds on Cotter Reservoir, though generally the highest abundances were observed in the warmer months (November – March). Great cormorants were almost always the most abundant species present. All three species of piscivorous birds (Great cormorant, Little black cormorant and Little pied cormorant) were found to be more abundant in the most upstream fifth of the Cotter Reservoir. The increased abundances during warmer months together with the preference for the upstream fifth of the Cotter Reservoir pose a significant predation risk to Macquarie perch as they move between the reservoir and the river to spawn from late spring to summer.

Rainbow trout were a common capture in all three reservoirs on the Cotter River, with the reference reservoirs of Corin and Bendora having a higher abundance compared to Cotter Reservoir. The low abundance of trout in Cotter Reservoir is likely a result of the prolonged drought, the lower elevation of Cotter Reservoir (hence, higher water temperatures), and high sediment load from the bushfire-affected commercial forestry plantations. However, it is likely that trout abundance will increase in Cotter Reservoir during filling, as thermal refuge habitat and food resources increase. Abundances of Rainbow trout were consistent across years for Corin and Bendora Reservoirs, but Cotter Reservoir exhibited some variability with a relatively low capture rate in 2011. The relatively constant trout abundances observed suggest that the current survey design is adequate for monitoring adult trout populations. Rainbow trout abundance (as determined by fyke net captures and backpack electrofishing) was relatively stable throughout the study period in the Cotter River upstream of the Cotter Reservoir.

As well as threatened native fish species and their potential predators, the baseline abundance of other alien fish species present in the Cotter Reservoir were also assessed. Goldfish were found to fluctuate in abundance in Cotter Reservoir between years, most likely due a difference in the sampling time (affecting water temperature) and the water level of the Cotter Reservoir. Sampling at the start of April rather than May is recommended as the water temperature is warmer and is most likely associated with greater movements (and catchability) of Goldfish in fyke nets. Abundance of Oriental weatherloach in Cotter Reservoir was low across all three years. No Goldfish or Oriental weatherloach were detected at the reference reservoir. The current study recommends that an alternative reference reservoir be sought that contains comparable populations of Goldfish and Oriental weatherloach to Cotter Reservoir.

Overall the study design appears to be adequate to assess each of the 12 management questions and will provide a valuable resource for detecting change and informing management decisions once the ECD starts to fill. The three years of baseline assessment data collection and preliminary analysis has identified a small number of modifications that will be beneficial in the longer term by providing more robust population assessment and comparisons. The assessment program should continue to gather baseline data as the ECD fills as this is an opportunity that is rarely available to collect information on biotic response to terrestrial inundation.

RECOMMENDATIONS

1.1 Continue with the current fyke netting sampling design for assessing abundance of YOY and juvenile Macquarie perch to determine if a change occurs as a result of ECD.
1.2 Continue with current gill netting sampling design for assessing abundance of adult Macquarie perch to determine if a change occurs as a result of ECD.
1.3 Investigate suitability of boat electrofishing to compliment gill net captures of adult Macquarie perch as sampling during high water using current technique was ineffective in capturing adult Macquarie perch.

2.1 Continue with current sampling methodology for assessing recruitment of YOY Macquarie perch in the ECD to determine if a change occurs as a result of ECD.
2.2 Add a second reference site in the upper Murrumbidgee catchment for assessing recruitment of YOY Macquarie perch in the ECD. This will increase the power of detection as currently the reference site is relatively variable in the level of recruitment between years.

3.1 The current sampling design should remain unchanged for assessing significant changes in the abundance, distribution and size composition of adult trout in the ECD.

4.1 Explore alternative trout collection techniques (angling) to assess predation on Macquarie perch larvae if high flows prevent backpack electrofishing. This will ensure that sampling seasons are not missed as this reduces the power of the sampling design to detect change over the course of seasons and years.
4.2 Further develop genetic detection capabilities to detect Macquarie perch in the diet of trout.
4.3 In addition to the spring trout sample collected for Q7, collect a separate sample of trout to examine potential predation on Macquarie perch. This will spread the risk of environmental conditions preventing both samples from being collected.

5.1 The current sampling design should remain unchanged for assessing abundance, distribution and species composition of piscivorous birds in the ECD.

6.1 The current sampling design should remain unchanged for assessing abundance and size composition of trout in the Cotter River upstream of the ECD.

7.1 The current sampling design should remain unchanged for assessing levels of predation on Two-spined blackfish by trout in the Cotter River upstream of the ECD.

8.1 To increase the robustness of the sampling regime to detect a true change in the abundance of Macquarie perch upstream and downstream of Vanitys Crossing, the addition of a second riverine reference site is recommended (see recommendation 2.2).

8.2 Backpack electrofishing should continue to be deployed in assessing the abundance and distribution of Macquarie perch upstream and downstream of Vanitys Crossing, but should not be used to compare abundance of Macquarie perch between sites and years as the number of Macquarie perch captured by this method is very low. (This technique is still of use as an accompaniment to fyke netting to determine changes in distribution (ie presence or absence) of Macquarie perch at a site between years).

11.1 The current sampling regime should continue to assess whether Two-spined blackfish will establish a reproducing population in the ECD and will they persist in the newly inundated section of the river.

12.1 The use of a second reference impoundment should be explored that has comparable abundances of Goldfish and Oriental weatherloach to Cotter Reservoir (Yerrabi Pond, Gungahlin, is the suggested choice). To date, sampling of the current reference site has failed to capture either of these alien species and therefore is a poor reference site.

12.2 Sampling for Goldfish and Oriental weatherloach should be conducted in late March/early April in future years before water temperatures drop. Once water temperatures drop, these species are less active and are therefore less likely to be captured by passive gear types like fyke nets (the method used in this study).

INTRODUCTION

Prolonged drought in the ACT resulted in the recommissioning of Cotter Dam and the augmentation of the Cotter Reservoir from ~4 GL to 78GL capacity. A long-term assessment program to determine the impacts of the enlarged Cotter Dam (ECD) on threatened fish and other selected fauna has been commissioned by ACTEW Corporation, and is being undertaken by the University of Canberra. Phase 1 of the assessment program covers the years 2010-2012 inclusive. It is a wide-ranging assessment, focussing not only on the threatened species populations themselves, but also avian and fish predators and introduced competitors. Following the preparation of a scope of works (Lintermans 2009) and statistical review of the draft proposed methodology and available background data (Robinson 2009), the final assessment program was designed (Lintermans and Broadhurst 2010). The assessment program is not intended to examine specific interventions that have been undertaken as part of the ECD fish management program (ACTEW Corporation 2010; Lintermans 2012).

This report presents the findings from the Phase 1 sampling to develop a baseline to assess future potential impacts on threatened fish from the construction, filling and operation of the ECD. The report is structured to address the 12 questions identified in the final sampling design (Lintermans and Broadhurst 2010):

1) Will there be significant changes in the abundance of Macquarie perch in the ECD (Young-of-Year, juveniles and adults)
2) Will there be a significant change in annual recruitment in the Macquarie perch population in the ECD relative to a reference site (Kissops Flat)?
3) Will there be significant changes in the abundance, distribution and size composition of adult trout in the ECD?
4) What are the levels of predation on Macquarie perch larvae and juveniles by trout in the ECD and river upstream?
5) Will there be significant changes in the abundance, distribution and species composition of piscivorous birds in the ECD?
6) Will there be significant changes in the abundance and size composition of trout in the Cotter River upstream of the ECD?
7) Will there be a significant increase in the levels of predation on Two-spined blackfish by trout in the Cotter River upstream of the ECD?
8) Will there be significant changes in the abundance and distribution of the Macquarie perch population in the Cotter River above and below Vanitys Crossing?
9) Will macrophyte beds re-establish in the enlarged ECD?*
10) Will translocated Macquarie perch populations survive the initial translocation procedure and reproduce?[#]
11) Will Two-spined blackfish establish a reproducing population in the ECD and will they persist in the newly inundated section of the river?
12) Will there be significant changes in the abundance and distribution of Goldfish and Oriental weatherloach in the ECD?

*Question 9 cannot be determined until the ECD is established.

Question 10 is being addressed by a separate project (Lintermans 2011).

METHODS

The assessment was primarily undertaken in the Cotter River catchment (Table 1 & Figure 1) (including its impoundments), but also included several sites outside the Cotter Catchment which acted as independent reference sites (Table 1 & Figure 2).

Table 1. List of sites and the monitoring questions addressed.

Catchment	Site	Question addressed
Cotter River - regulated	Cotter Reservoir	1–5, 9, 11, 12.
	Bracks Hole	4, 6–8, 11.
	Vanitys Crossing	4, 6–8, 11.
	Spur Hole	4, 6–8, 11.
	Pipeline Rd. Crossing	4, 6–8, 11.
	Burkes Ck. Crossing	4, 6–8, 11.
	Downstream of Bendora Dam	4, 6–8, 11.
	Bendora Reservoir	3.
Cotter River - unregulated	Corin Reservoir	3.
	Gallipoli Flat	7, 10.
	Above Gallipoli Flat	10
	Upstream of Cribbs Ck.	10
	Cotter Hut	6, 7, 11.
Murrumbidgee River	Kissops Flat	1, 2, 8, 12.
Goodradgibee River	Micalong Ck.	6.
Ginninderra Ck.	Lake Ginninderra	12.

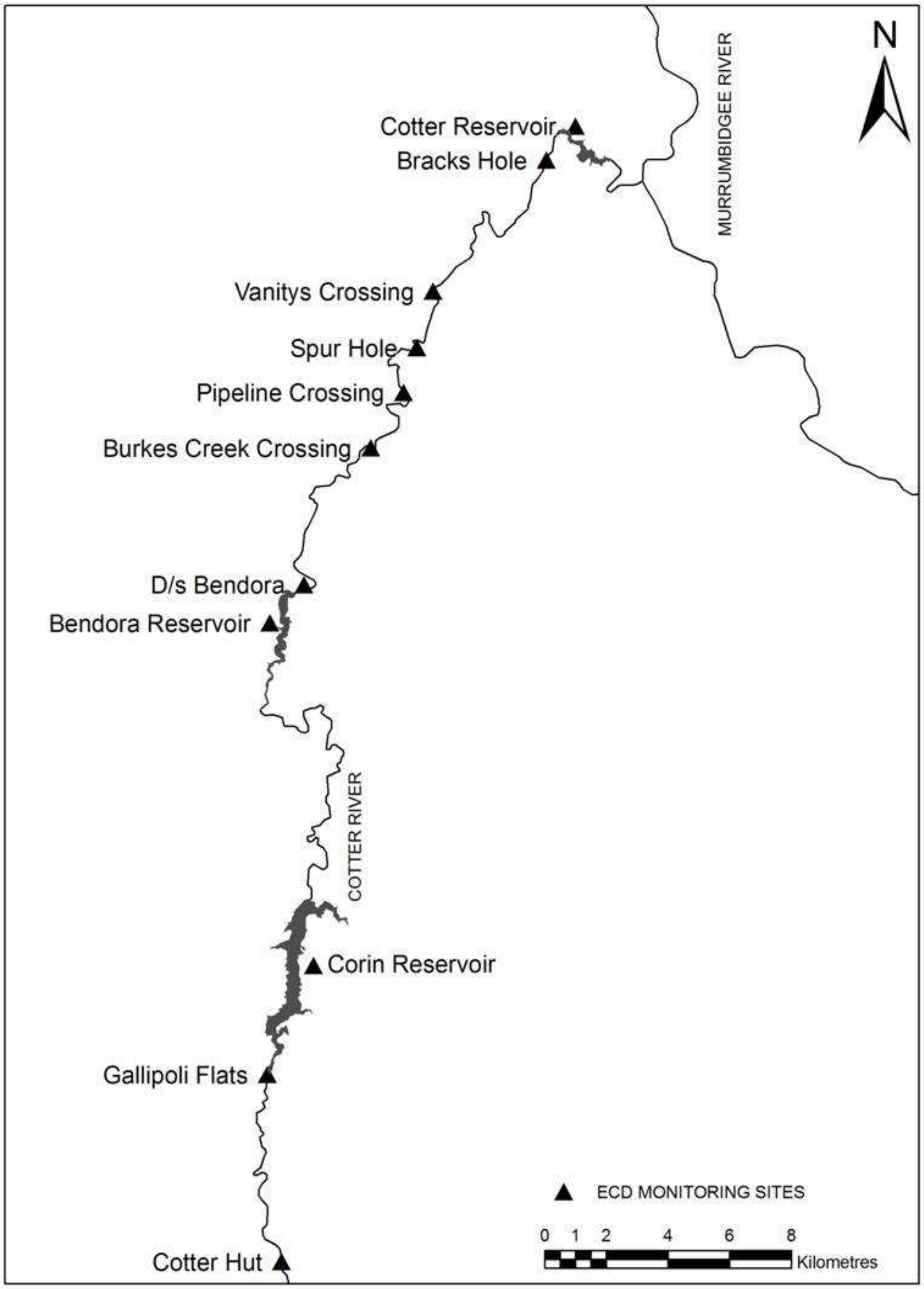

Figure 1. Location of study sites within the Cotter River catchment.

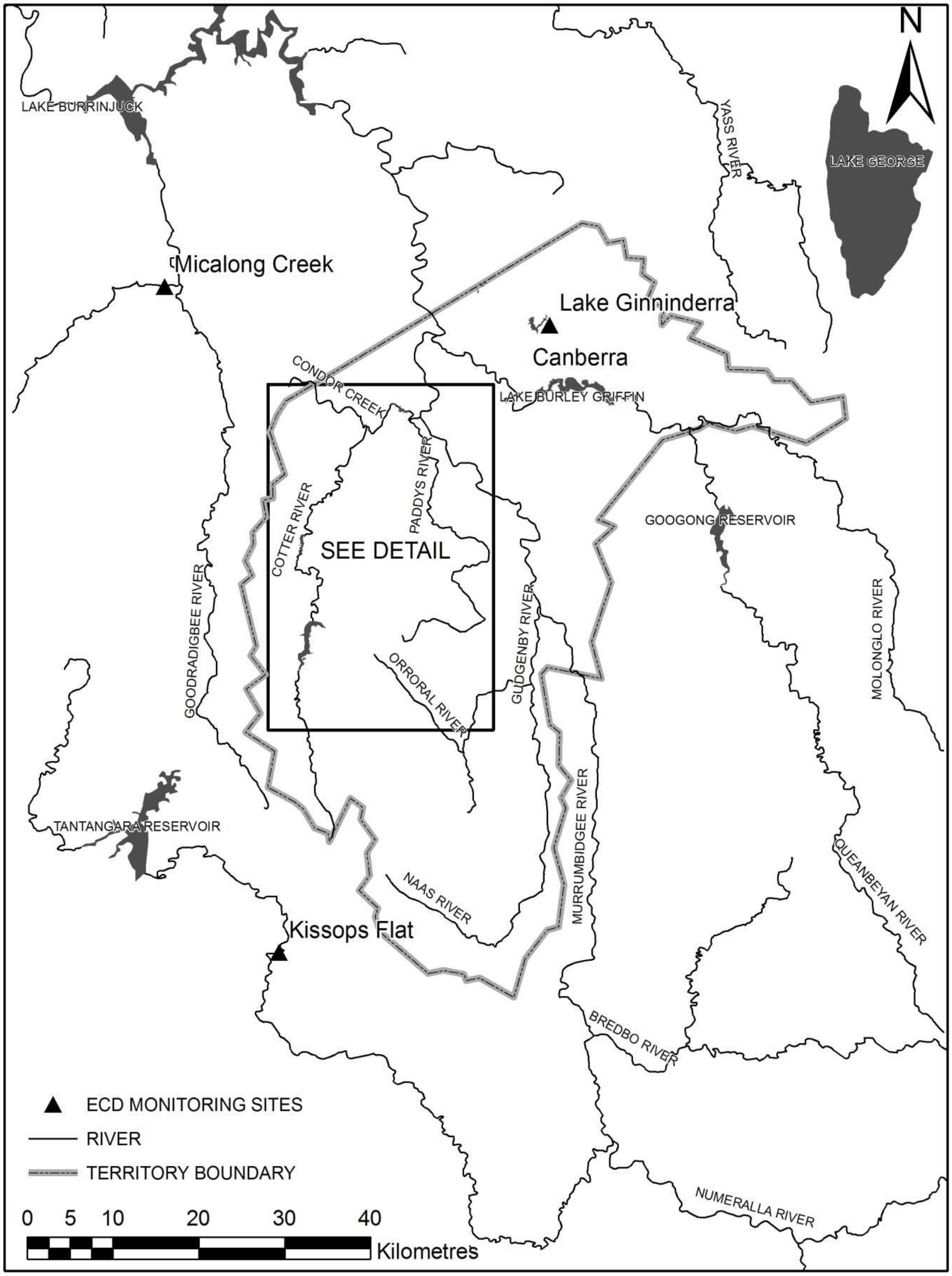

Figure 2. Extent of study sites outside the Cotter River Catchment.

SUMMARY OF ACTIVITIES UNDERTAKEN

In both 2010 and 2011, high water flows or high turbidity prevented some sampling from occurring at some sites. As a result of flooding in spring 2010, a sample of trout stomach contents was not able to be obtained for this season (Table 2). In 2011, a summer electrofishing sample was not able to be collected from Micalong Ck. due to high flows and high turbidity (Table **3**). Micalong Ck and Kissops Flat were not able to be electrofished in 2012 due to high river flows and associated high turbidity (

Table **4**). High flows in 2012 also prevented Micalong Ck from being sampled using fyke nets (

Table **4**).

These gaps in sampling effort are unforeseeable, and likely to happen in many years as a result of climatic events. As much of the sampling is scheduled towards the end of some seasons to answer multiple management questions and reduce sampling costs (e.g. spring to collect data for questions 4, 7 & 8), it is not possible to return to sites to fill sampling gaps in the same season. For example, prolonged high flows in November means that any return visit would have to be in summer). Statistical advice is being sought on the potential ramifications of such sampling gaps, and it is recommended that the sampling schedule be amended to reduce the likelihood of such occurrences (see recommendations for Question 4).

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Table 2. List of completed assessment activities conducted in 2010.

Site	Fyke netting	Gill netting	Summer e/fishing	Autumn e/fishing	Winter e/fishing	Spring e/fishing
Cotter Reservoir	Yes	Yes	N/A	N/A	N/A	N/A
Bracks Hole	Yes	N/A	Yes	Yes	Yes	No[1]
Vanitys Crossing	Yes	N/A	Yes	Yes	Yes	No[1]
Spur Hole	Yes	N/A	Yes	Yes	Yes	No[1]
Pipeline Road Crossing	Yes	N/A	Yes	Yes	Yes	No[1]
Burkes Creek Crossing	Yes	N/A	Yes	Yes	Yes	No[1]
Downstream Bendora	Yes	N/A	Yes	Yes	Yes	No[1]
Bendora Reservoir	Yes	Yes	N/A	N/A	N/A	N/A
Corin Reservoir	Yes	Yes	N/A	N/A	N/A	N/A
Gallipoli flat	N/A	N/A	Yes	Yes	Yes	No[1]
Cotter Hut	Yes	N/A	Yes	Yes	Yes	No[1]
Micalong Ck	Yes	N/A	Yes	N/A	N/A	N/A
Kissops Flat - M'bidgee River	Yes	N/A	Yes	N/A	N/A	N/A

[1] Site was not able to be electrofished as a result of spring flooding in the Cotter River Catchment.

Table 3. List of completed assessment activities conducted in 2011.

Site	Fyke netting	Gill netting	Summer e/fishing	Autumn e/fishing	Winter e/fishing	Spring e/fishing
Cotter Reservoir	Yes	Yes	N/A	N/A	N/A	
Bracks Hole	Yes	N/A	No[1]	Yes	Yes	
Vanitys Crossing	Yes	N/A	Yes	Yes	Yes	

Spur Hole	Yes	N/A	Yes	Yes	Yes
Pipeline Road Crossing	Yes	N/A	Yes	Yes	Yes
Burkes Creek Crossing	Yes	N/A	Yes	Yes	Yes
Downstream Bendora	Yes	N/A	Yes	Yes	Yes
Bendora Reservoir	Yes	Yes	N/A	N/A	N/A
Corin Reservoir	Yes	Yes	N/A	N/A	N/A
Gallipoli flat	N/A	N/A	Yes	Yes	Yes
Cotter Hut	Yes	N/A	Yes	Yes	Yes
Micalong Ck Reserve	Yes	N/A	No[1]	N/A	N/A
Kissops Flat - M'bidgee River	Yes	N/A	N/A	Yes	N/A

[1] Site was not able to be electrofished as a result of high flow and turbidity.

Table 4. List of completed assessment activities conducted in 2012.

Site	Fyke netting	Gill netting	Summer e/fishing	Autumn e/fishing	Winter e/fishing	Spring e/fishing
Cotter Reservoir	Yes	Yes	N/A	N/A	N/A	N/A
Bracks Hole	Yes	N/A	Yes	Yes	Yes	Yes
Vanitys Crossing	Yes	N/A	Yes	Yes	Yes	Yes
Spur Hole	Yes	N/A	Yes	Yes	Yes	Yes
Pipeline Road Crossing	Yes	N/A	Yes	Yes	Yes	Yes
Burkes Creek Crossing	Yes	N/A	Yes	Yes	Yes	Yes
Downstream Bendora	Yes	N/A	Yes	Yes	Yes	Yes
Bendora Reservoir	Yes	Yes	N/A	N/A	N/A	N/A
Corin Reservoir	Yes	Yes	N/A	N/A	N/A	N/A
Gallipoli flat	N/A	N/A	Yes	Yes	No[1]	Yes
Cotter Hut	Yes	N/A	Yes	Yes	No[1]	Yes
Micalong Ck	No[1]	N/A	No[1]	N/A	N/A	N/A
Kissops Flat - M'bidgee River	Yes	N/A	N/A	No[1]	N/A	N/A

[1] Site was not able to be electrofished as a result of high flow and turbidity

RESULTS

General results

A total of 5554 fish from 13 species were captured across the 3 years of sampling (Table 5). Macquarie perch was the most abundant species captured in reservoir environments, and Two-spined blackfish in riverine environments.

Table 5. Total number of each species of fish captured in reservoirs and in rivers by each sampling technique.

	Reservoirs		Riverine		
Species	Gill nets	Fyke nets	E/fishing	Fyke nets	**Total**
Macquarie perch	113	872	65	731	**1781**
Two-spined blackfish	-	74	1273	341	**1688**
Rainbow trout	323	25	1123	40	**1511**
Goldfish	1	110	64	97	**272**
Oriental weatherloach	-	5	31	6	**42**
Brown trout	1	-	43	4	**48**
Golden perch	-	7	-	-	**7**
Murray cod	-	4	-	-	**4**
Trout cod	11	-	4	2	**17**
Redfin perch	-	34	2	10	**46**
Carp	-	-	28	53	**81**
Eastern gambusia	-	-	47	-	**47**
Mountain galaxias	-	-	10	-	**10**
Total	**449**	**1131**	**2690**	**1284**	**5554**

The results are presented individually below for each management question.

1) Will there be significant changes in the abundance of Macquarie perch in the ECD (Young-of-Year, juveniles and adults)

A range of potential threats (for example, loss of habitat, interactions with alien fish species, predation by cormorants etc) will potentially impact on the Macquarie perch population in the ECD. Monitoring of changes in the population size of major life-history phases of Macquarie perch (Young of year, juveniles, and adults) will provide an early indication that additional mitigation or further information on specific threats is required.

Sampling design

Adults

Adult Macquarie perch in Cotter Reservoir were captured by gill nets in autumn each year. Ten gill nets, comprising three each of 76 mm & 102 mm stretch mesh (100 mesh deep), 2 x 127 mm (50 mesh deep), 1 each of 76 mm & 102 mm (50 mesh deep) were set from ~1500 – ~ 2230 hrs (gill nets checked hourly). The reservoir is stratified into five sections with two gill nets set per section, with mesh types randomly allocated each night. Netting is conducted for five nights. Macquarie perch are returned alive at the site of capture.

Sub-adults and juveniles

To assess abundance of sub-adult and juvenile Macquarie perch, Cotter Reservoir and a riverine reference site (Kissops Flat) are sampled using fyke nets in autumn each year. Ten fyke nets (single-wing, 13 mm stretched mesh) are set on the first night at both sites. At Cotter Reservoir, the 10 fyke nets for the first nights sampling are set in the downstream half of the reservoir (typically the area of the reservoir with the highest abundance of sub-adults and juveniles). On the second night of sampling, to minimise sampling cost by addressing multiple management questions (Q1 and Q12), 12 nets are set at each site, and these 12 nets are set at fixed locations around the entire reservoir. However for Q1 only data from the most downstream 10 nets was used.

Results

Adult numbers

In autumn 2010 over five nights of sampling in Cotter Reservoir (27/04/2010, 29/04/2010, 04/05/2010, 05/05/2010 and 06/05/2010), a total of 46 adult Macquarie perch were captured ranging in size from 207 – 407 mm total length (TL) (Figure 3).

In autumn 2011, the five nights of sampling (28/03/2011, 29/03/2011, 04/04/2011, 05/04/2011 and 06/04/2011) captured a total of 50 adult Macquarie perch ranging in size from 207 – 410 mm (TL) (Figure 4).

In autumn 2012, the five nights of sampling (02/04/2012, 03/04/2012, 04/04/2012, 02/05/2012 and 03/05/2012) resulted in a total of 18 adult Macquarie perch captured ranging in size from 210 – 416 mm TL (Figure 5). No adult Macquarie perch were caught on nights one and two with only a single individual caught on night three. During these three nights the water level was ~5 – 15 m above the full supply level of the reservoir (Figure 11).

The overall catch per unit effort (CPUE) of adult Macquarie perch in gill nets was similar between 2010 and 2011 but was considerably lower in 2012 (Figure 6).

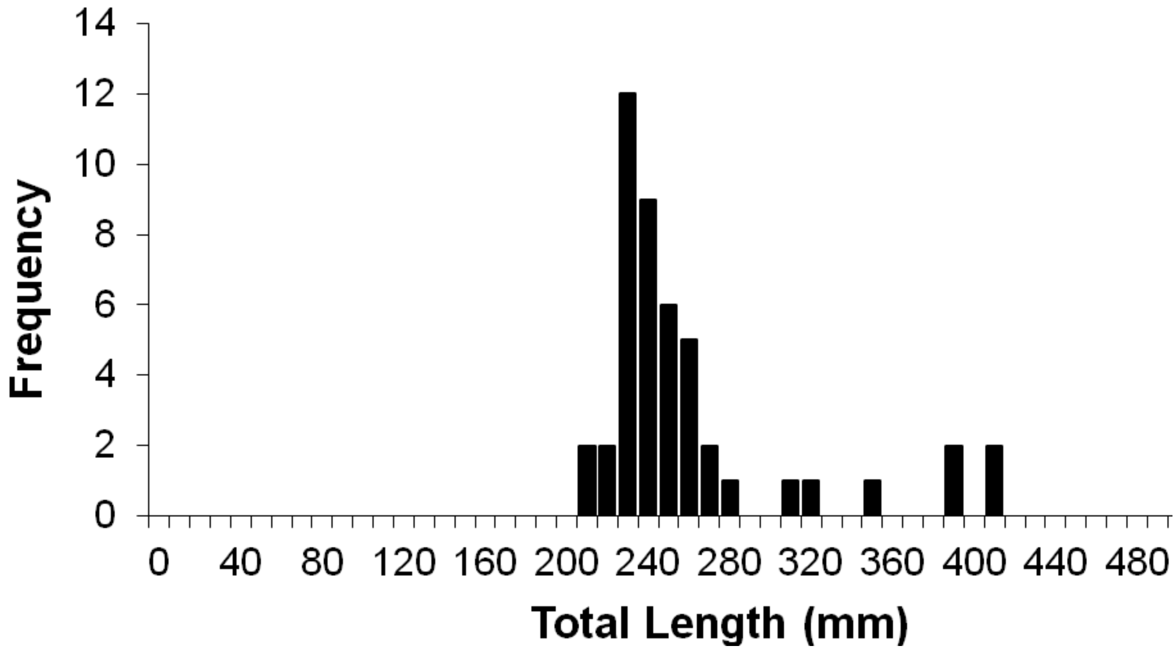


Figure 3. Length of Macquarie perch adults caught by gill nets from Cotter Reservoir over five nights sampling combined in autumn 2010.

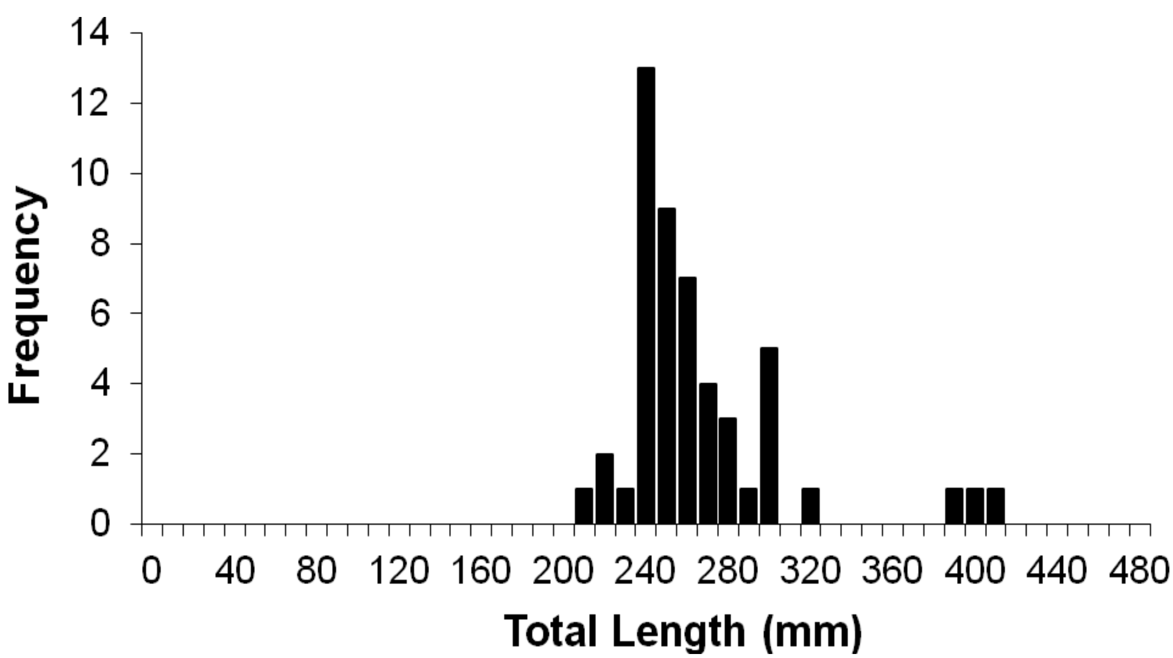


Figure 4. Length of Macquarie perch adults caught by gill nets from Cotter Reservoir over five nights sampling combined in autumn 2011.

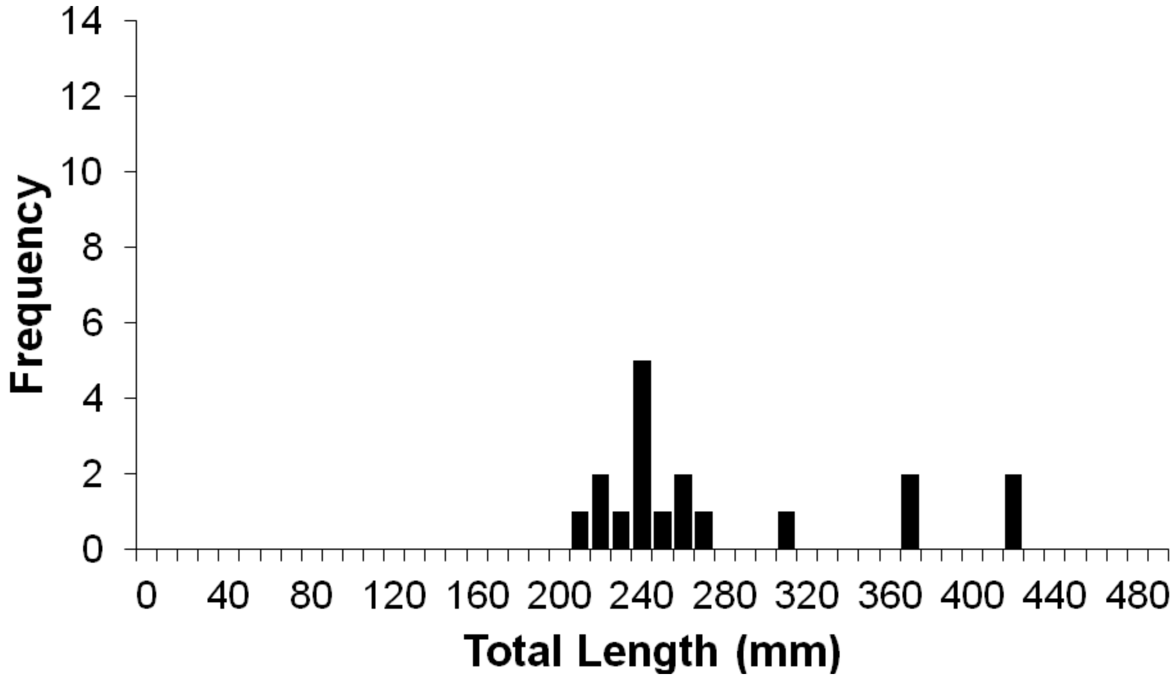


Figure 5. Length of Macquarie perch adults caught by gill nets from Cotter Reservoir over five nights sampling combined in autumn 2012.

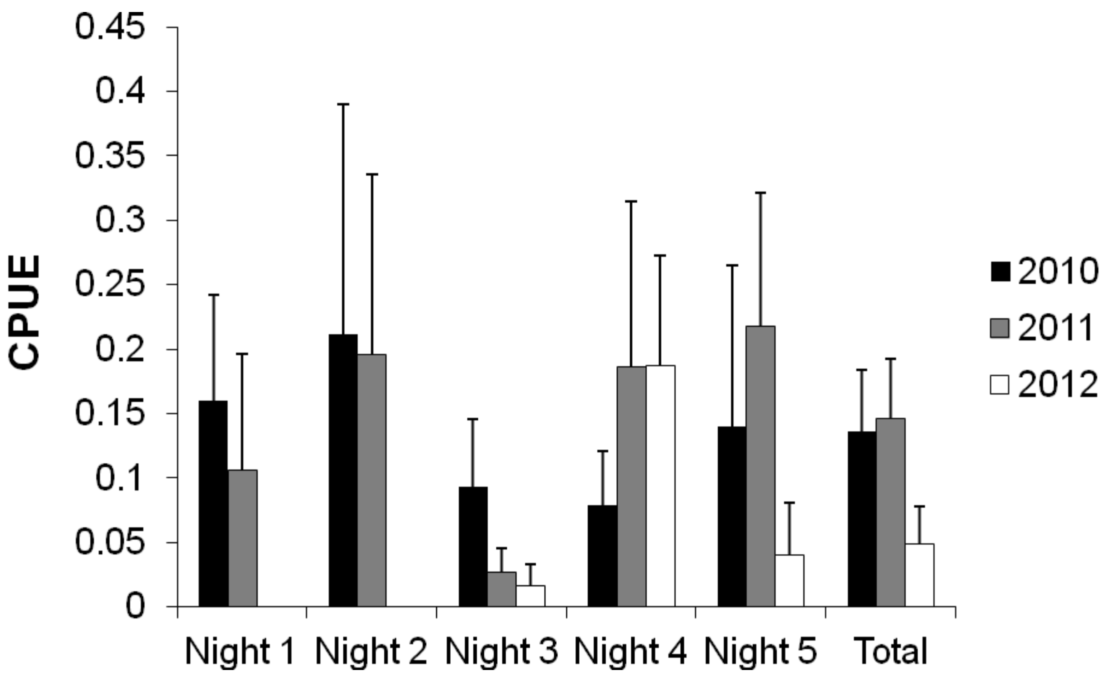


Figure 6. Mean (±SE) Macquarie perch captured per gill-net hour (CPUE) from Cotter Reservoir for each sampling night and total.

Young-of-Year and juveniles

Ten fyke nets were set per night for two nights in 2010 (29/04/2010 & 04/05/2010), 2011 (28/03/2011 & 04/04/2011) and 2012 (02/04/2012 and 02/05/2012) in Cotter Reservoir. A total of 523 Macquarie perch were caught in 2010 ranging in size from 45 – 263 mm TL (Figure 7). The catch data was dominated by two year classes; young of the year (YOY) (50 – 100 mm) and age 1+ fish (110 – 180 mm).

In 2011 a total of 192 Macquarie perch were caught ranging in size from 49 – 323 mm TL (Figure 8). The numbers of YOY (50 – 100 mm) Macquarie perch caught in 2011 (Figure 8) were considerably lower compared to 2010 (Figure 7) and the total catch in 2011 was only 37% of the total catch from 2010.

In 2012 a total of 98 Macquarie perch were caught ranging in size from 47 – 222 mm TL (Figure 9). The catch was dominated by YOY Macquarie perch (Figure 9), which was higher than 2011 but significantly lower than 2010. Only three individuals were caught in the first nights sampling (02/04/2012) at which time the dam level was ~5 – 15 m above full supply level (Figure 11).

Catch per unit effort of Macquarie perch (both all sizes and juveniles) was much lower in 2011 and 2012 compared to 2010 (

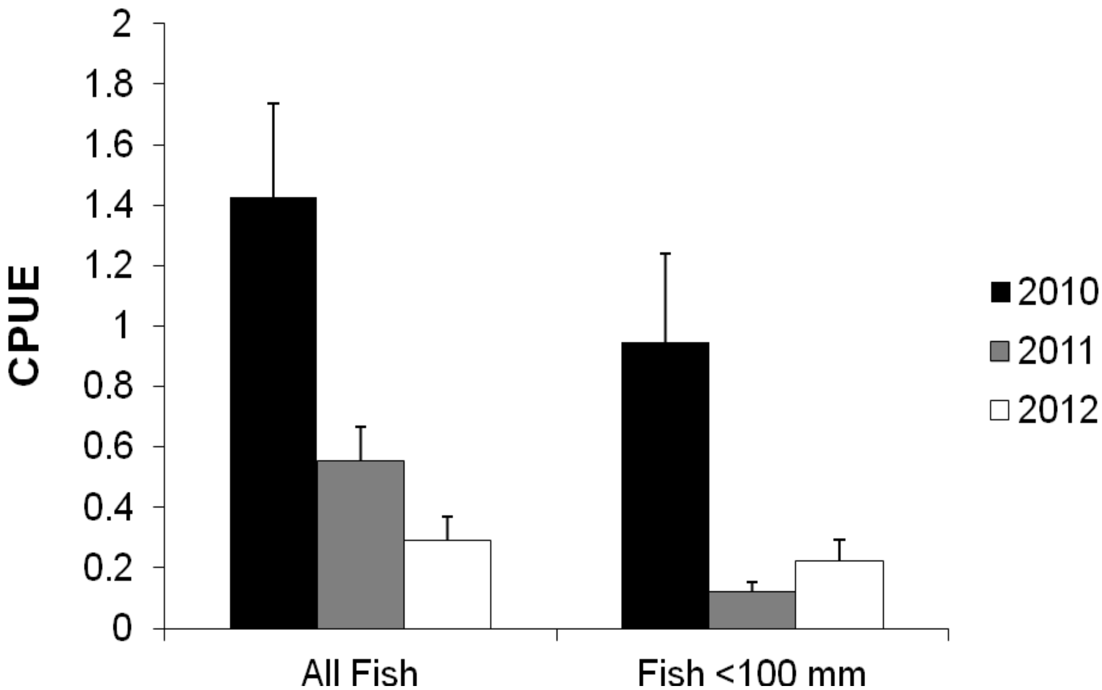


Figure 10). However, the fyke net catch from 2010 was exceptionally high compared to fyke net sampling in other years (pre 2010, Lintermans unpublished data). Young of year CPUE was higher in 2012 than 2011 indicating successful recruitment from the 2011 spawning season (Figure 10).

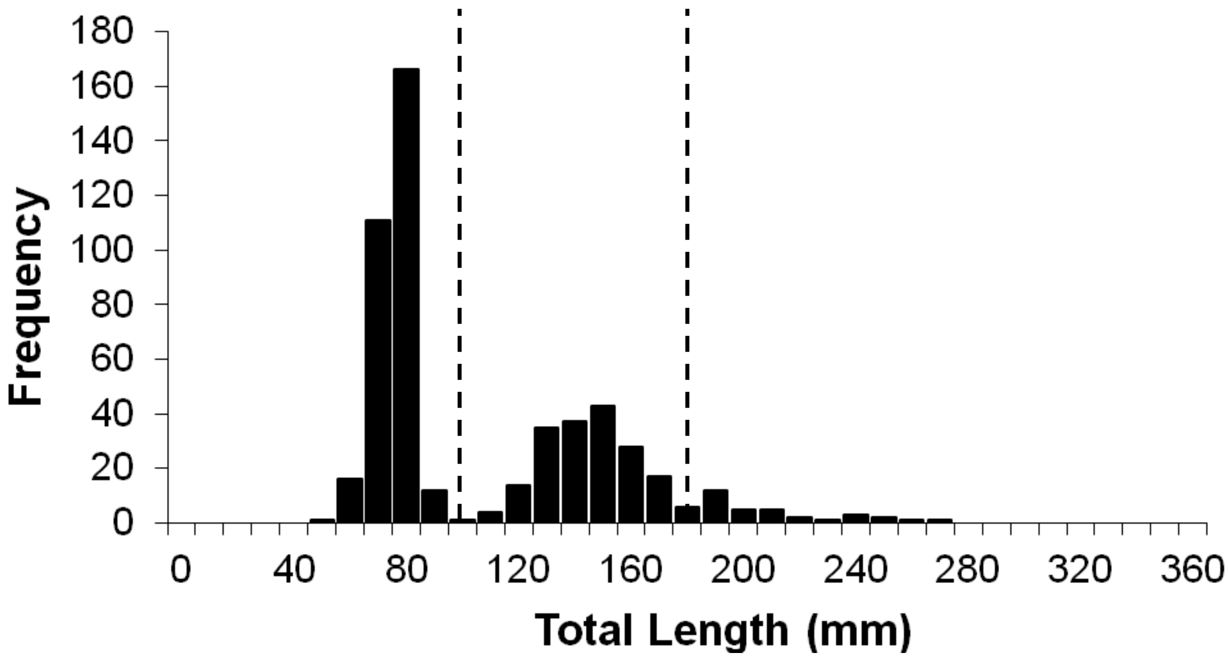


Figure 7. Length of Macquarie perch caught by fyke net from Cotter Reservoir over two nights sampling combined in autumn 2010. Dashed line indicates YOY and juvenile cohorts.

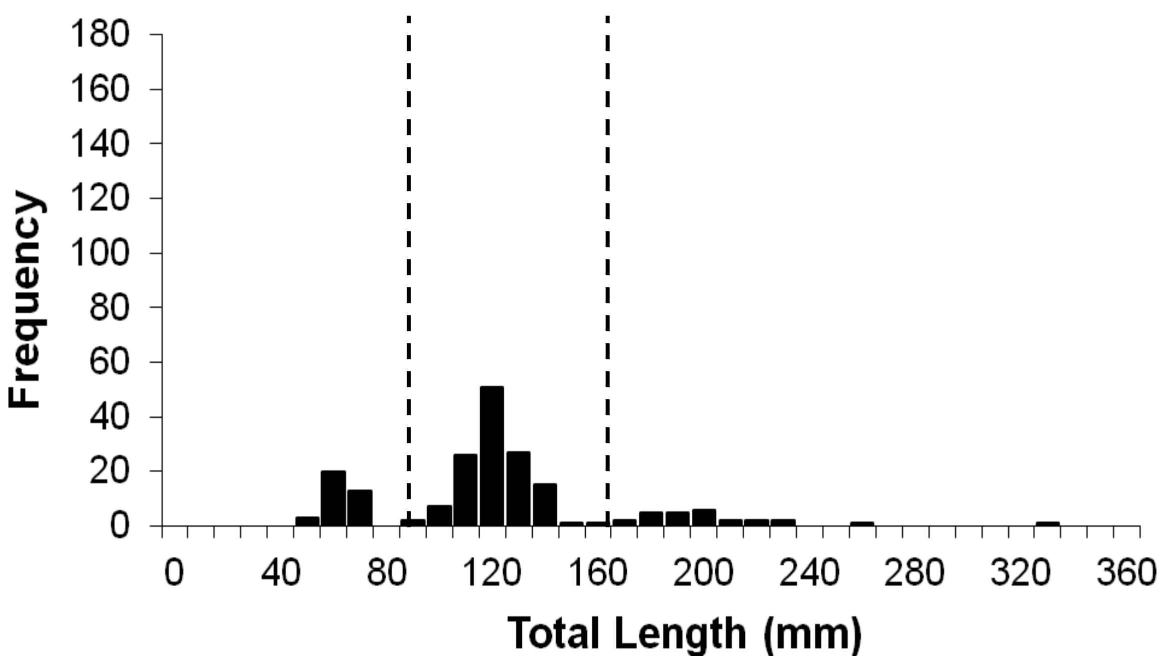


Figure 8. Length of Macquarie perch caught by fyke net from Cotter Reservoir over two nights sampling combined in autumn 2011. Dashed line indicates YOY and juvenile cohorts.

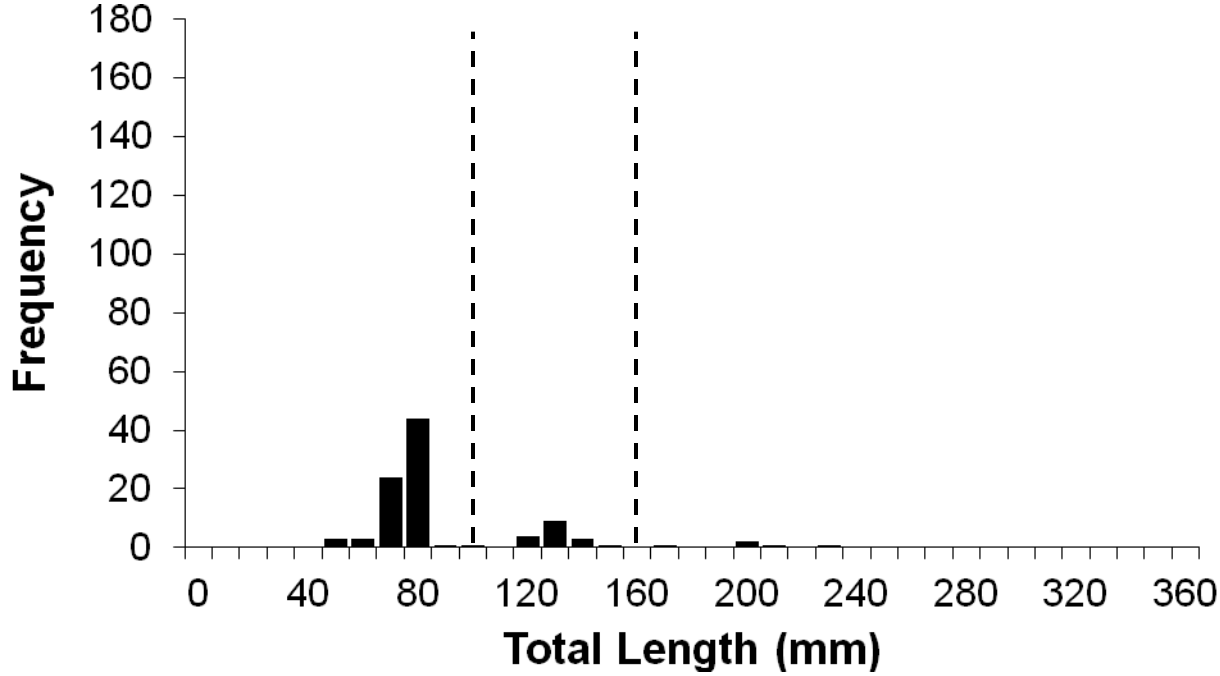


Figure 9. Length of Macquarie perch caught by fyke net from Cotter Reservoir over two nights sampling combined in autumn 2012. Dashed line indicates YOY and juvenile cohorts.

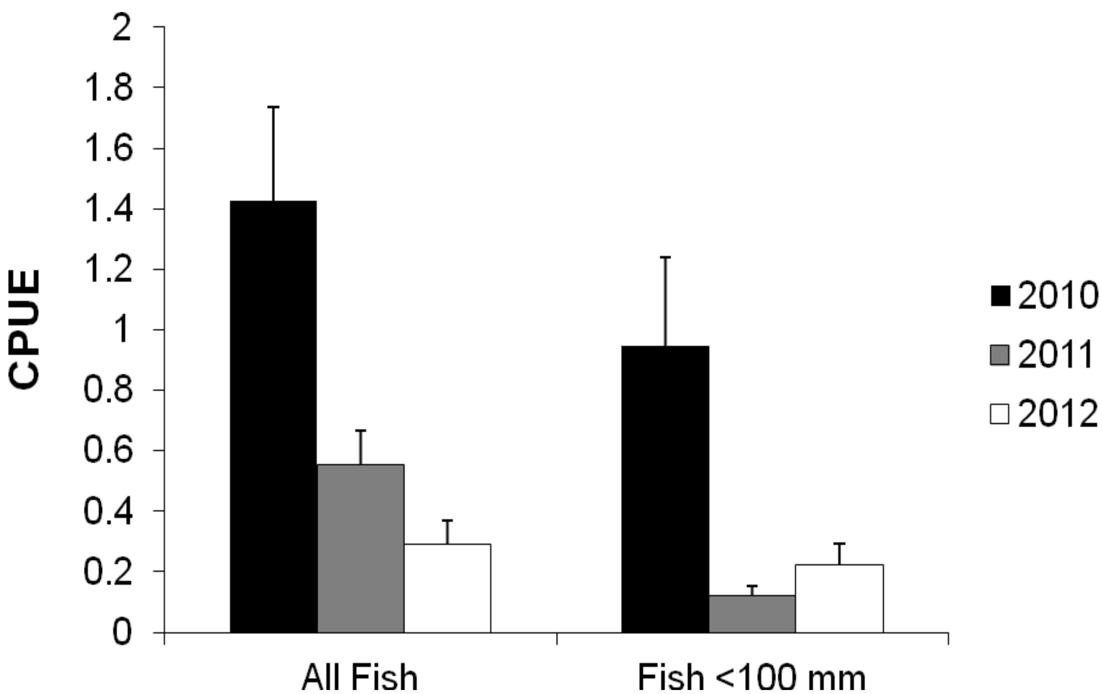


Figure 10. Mean (± SE) number of Macquarie perch captured per fyke-net hour (CPUE) from Cotter Reservoir.

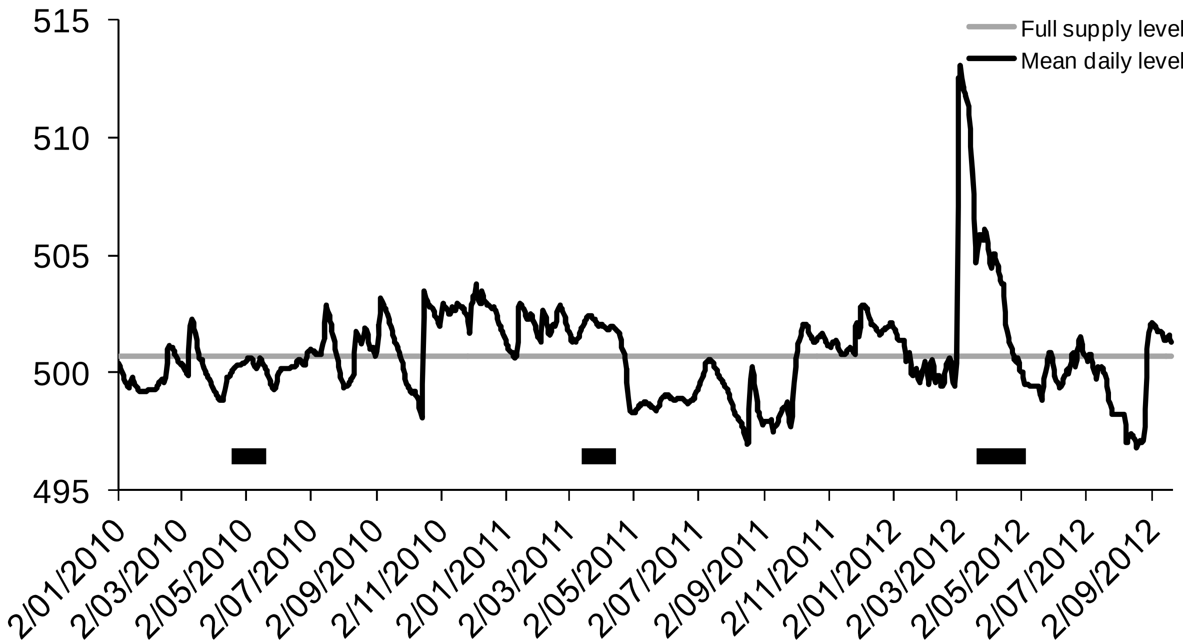


Figure 11. Cotter Reservoir water level during the monitoring period. Grey line indicates full supply level, black line is the mean daily level. Thick black lines indicate times of gill netting in Cotter Reservoir.

Discussion

The abundance of adult Macquarie perch is tracking that recorded in previous samplings of the reservoir conducted in 2008-09 (Lintermans *et al.* 2010) (**Error! Reference source not found.**) and provides a five year baseline against which to measure future changes.

Table 6. Number of adult Macquarie perch captured in gill nets per night in Cotter Reservoir from 2008 – 2012 (2008 and 2009 data taken from (Lintermans et al. 2010) (Note: only three nights of sampling were undertaken in 2009).

Sample (night)	2008	2009	2010	2011	2012
1	13	11	12	7	0
2	8	11	14	14	0
3	16	2	6	2	1
4	10	-	5	13	13
5	12	-	9	13	3

No adult Macquarie perch were captured in gill nets in the first two of the five nights of sampling and only a single individual caught on night three when the reservoir was at high water level (5 – 15 m above the old FSL) in early 2012 (Figure 11). This was potentially because of the difficulty in setting gill nets in the near-shore habitats around the reservoir, with newly inundated terrestrial vegetation preventing the effective deployment of nets. The final two nights of sampling demonstrated that adult fish were present, but detecting them was difficult using gill nets. This could be problematic as sampling during this high-water event would closely resemble sampling conditions during the filling phase and early operation phase for the enlarged reservoir. The result may be low or nil catches of adult Macquarie perch, even though their abundance may not have altered. In order to counter this possible false negative (nil catch, but population present) another sampling technique directed at adult Macquarie perch should be explored. We propose that boat electrofishing of edge habitat be conducted one week prior to the 2013 gill netting in Cotter Reservoir. Utilising both techniques will allow some comparison of catches (both catch rates and the length frequency of adults captured) between the two techniques which will allow some transfer between the techniques in the future.

When the two nights of sampling conducted during high-water are removed, catches of adult Macquarie perch in gill nets are comparable with the other two years (quite consistent catches across years). In all three years the majority of Macquarie perch captured were between 200 and 300 mm TL. Male Macquarie perch reach sexual maturity at 150 – 200 mm TL and females at 300 mm TL (Lintermans 2007) so the majority of fish captured would be either sexually mature males or females that are approaching or have just reached sexual maturity.

Fyke net catches of YOY and juvenile Macquarie perch were much higher in 2010 compared to 2011 and 2012. The number of Macquarie perch captured in 2010 is high when compared to historical data when the median catch rate was 19.7 fish per fyke net (equating to a total catch of 197 individuals) (Lintermans, unpublished data). The large catch in 2010 was predominantly YOY (<100 mm TL) indicating that 2009 spawning and larval survival was very successful. Young-of-year (<100 mm) Macquarie perch were present in all three years indicating that annual spawning had occurred in 2009, 2010 and 2011. The catch rate in 2012 (total of 98 individuals) was low, when compared to the long-term median and may be related to the frequent alteration of reservoir water levels that have occurred as a result of the ECD construction program (low water level) and unseasonably high rainfall (high water level) in 2011.

Conclusion

During times when the reservoir water level was stable, the current sampling design for all age classes is adequate. During inundation of terrestrial vegetation, gill net catches of adult Macquarie perch were very low to nil and another sampling technique is required to either compliment or replace gill netting during such conditions.

Recommendations

1.4 Continue with the current fyke netting sampling design for assessing abundance of YOY and juvenile Macquarie perch to determine if a change occurs as a result of ECD.
1.5 Continue with current gill netting sampling design for assessing abundance of adult Macquarie perch to determine if a change occurs as a result of ECD.
1.6 Investigate suitability of boat electrofishing to compliment gill net captures of adult Macquarie perch as sampling during high water using current technique was ineffective in capturing adult Macquarie perch.

2) Will there be a significant change in annual recruitment in the Macquarie perch population in the ECD relative to a reference site (Kissops Flat)?

The continued health of the Macquarie perch population in the ECD is reliant on continued and regular recruitment. Monitoring of the Young-of-year life phase provides an instant measure of recruitment success from the previous spawning season. Whilst recruitment is not essential every year, a failure to recruit for a number of consecutive years is an early warning that there is a problem in the population. As Macquarie perch is a long-lived species, detection of problems at the recruitment stage gives maximum time to identify and address the problem.

Sampling design

Cotter Reservoir and a riverine reference site (Kissops Flat) were sampled in autumn each year. At each site, 10 fyke nets were deployed singly around the site. In Cotter Reservoir sampling is only conducted in the downstream half of the reservoir, as this is where most YOY fish are captured. Five fyke nets were set along each shoreline of the downstream half of the reservoir on the first night. On the second night, nets were reset avoiding re-sampling of the first nights net locations. At Kissops Flat fyke nets were set to sample the range of habitats present in the large pool present at the site. Netting was conducted for 2 non-consecutive nights at each site.

Results

Ten fyke nets per night were set for two nights in 2010 (29/04/2010 & 04/05/2010), 2011 (28/03/2011 & 04/04/2011), and 2012 (02/04/2012 and 02/05/2012) in Cotter Reservoir. A total of 523 Macquarie perch were caught in 2010 ranging in size from 45 – 263 mm TL (Figure 7). Of these 304 Macquarie perch were < 100 mm TL (YOY) (
Table **7**). A total of 192 Macquarie perch were caught in 2011 ranging in size from 49 – 323 mm TL (Figure 8) Of these 42 Macquarie perch were <100 mm TL (YOY) (
Table **7**). A total of 98 Macquarie perch were caught in 2012 ranging in size from 47 – 222 mm TL (Figure 9). Of these 75 Macquarie perch were <100 mm TL (YOY) (
Table **7**). The presence of YOY in each year indicates successful spawning from the previous season.

The reference site at Kissops Flat, on the upper Murrumbidgee River, was sampled for two nights in 2010 (20/04/2010 & 22/04/2010), 2011 (05/05/2011, 10/05/2011) and 2012 (10/04/2012 and 16/04/2012). A total of 27 Macquarie perch were caught in 2010 ranging in size from 46 – 281 mm TL (Figure 12). Ten Macquarie perch were captured with TL < 100 mm (
Table **7**). A total of 102 Macquarie perch were caught in 2011 ranging in size from 43 – 385 mm TL (Figure 13). Of these, 81 Macquarie perch were <100 mm TL (
Table **7**). A total of 143 Macquarie perch were caught in 2012 ranging in size from 38 – 413 mm TL (Figure 14). Of these, 137 Macquarie perch were <100 mm TL (
Table **7**). The presence of individuals < 100 mm TL in each year of sampling at Kissops Flat almost certainly indicates successful spawning from the previous season. The numbers of Macquarie perch captured at Kissops Flat each year is often too small to estimate annual cohorts of fish with absolute certainty, but individuals <100mm TL can confidently be assumed to be YOY, and usually comprise the majority of the catch of Macquarie perch at this site.

There was large variability in catch rates of YOY Macquarie perch between nights and years at both Cotter Reservoir and Kissops Flat (
Table **7**). Cotter Reservoir had a reduction in the catch rate of YOY Macquarie perch from 2010 to 2011, however had an increased in the catch rate from 2011 to 2012 (Figure 15). Kissops Flat recorded an opposite trend, with more YOY Macquarie perch caught per net hour in 2011 than in 2010 and another increase in 2012 compared to 2011 (Figure 15). The catch of fish < 100 mm TL in 2010 from Cotter Reservoir was exceptional, compared to previous years, but also demonstrates the high variability in catch rate of this size class within a year (i.e between individual nets).

Table 7. Catch per night of Macquarie perch <100 mm in fyke nets from Cotter Reservoir and Kissops Flat

		Cotter Reservoir	Kissops Flat
2010	Night 1	219	5
	Night 2	85	5
2011	Night 1	29	57
	Night 2	13	24
2012	Night 1	1	94
	Night 2	74	43

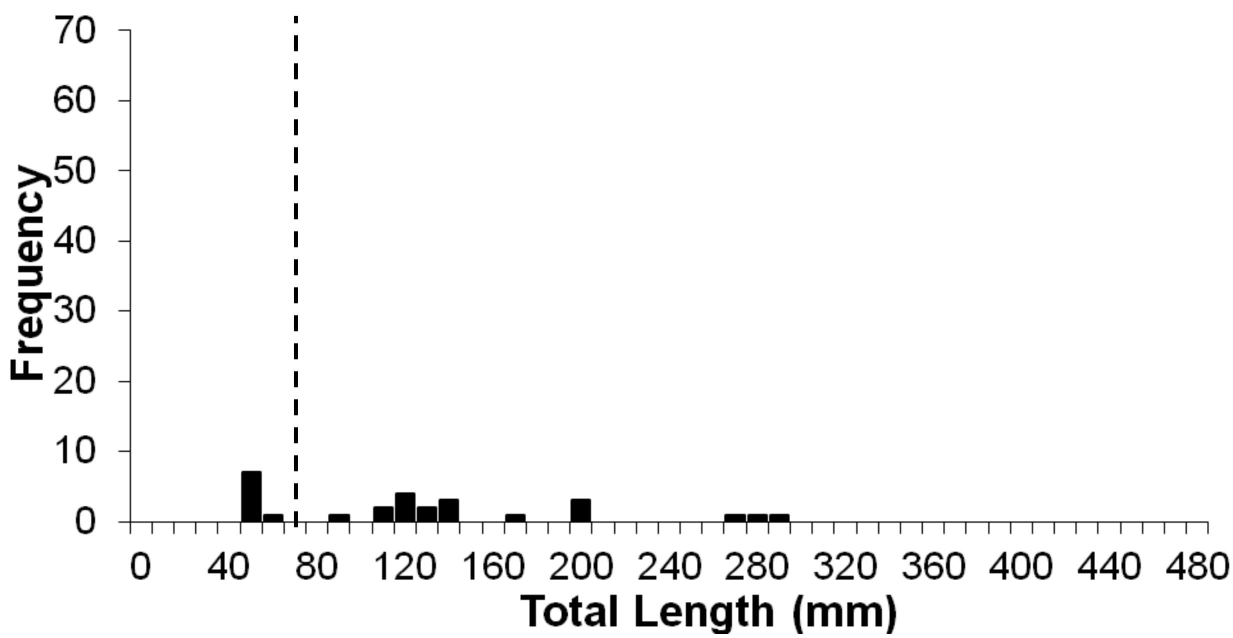


Figure 12. Length of Macquarie perch caught by fyke net from Kissops Flat over two nights sampling combined in April 2010. Dashed line indicates cut off for YOY.

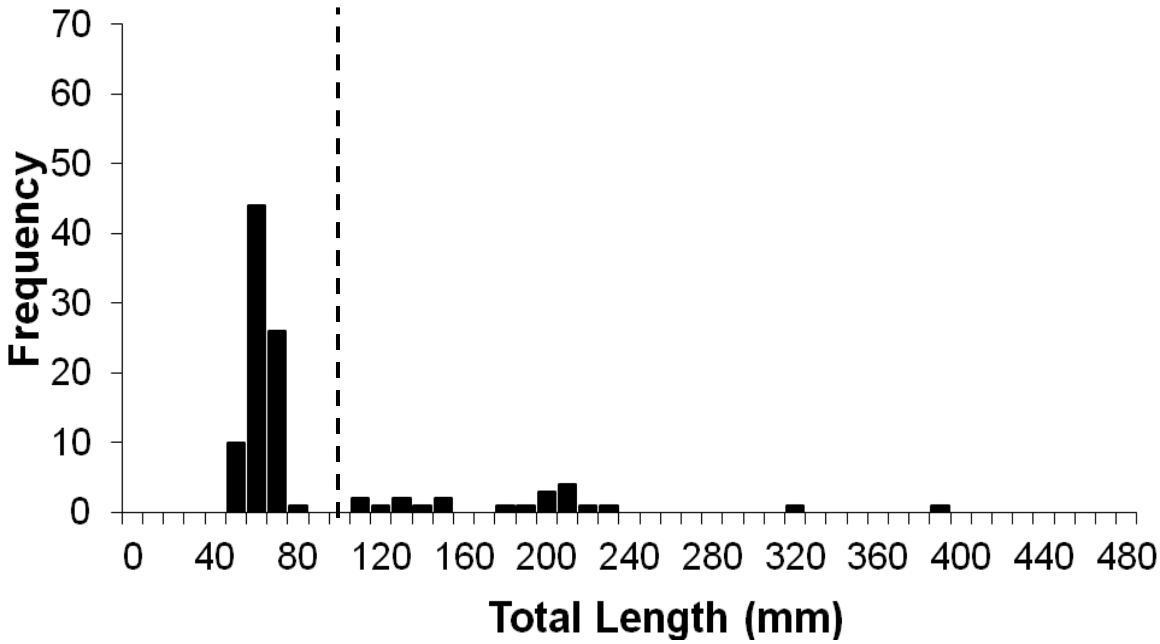


Figure 13. Length of Macquarie perch caught by fyke net from Kissops Flat over two nights sampling combined in May 2011. Dashed line indicates cut off for YOY.

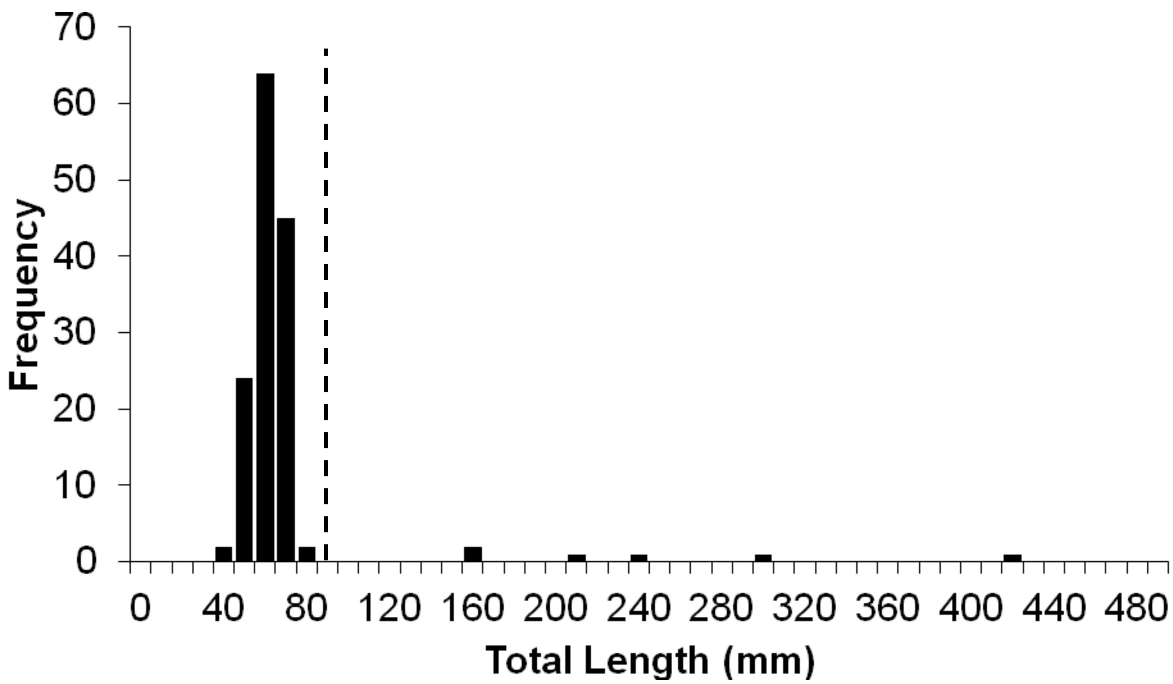


Figure 14. Length of Macquarie perch caught by fyke net from Kissops Flat over two nights sampling combined in April 2012. Dashed line indicates cut off for YOY.

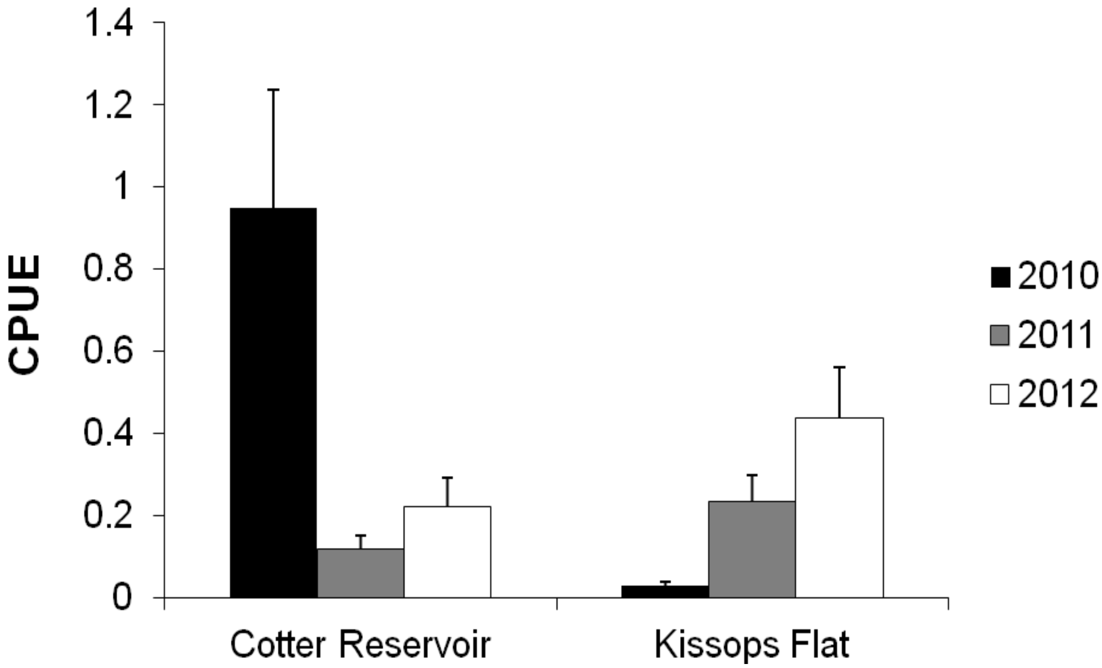


Figure 15. Mean (± SE) YOY Macquarie perch captured per fyke-net hour (CPUE) from Cotter Reservoir and Kissops Flat.

Discussion

Recruitment of YOY Macquarie perch (fish < 100 mm TL) was detected in all three years at both Cotter Reservoir and Kissops Flat. A very high number of YOY were present in 2010 at Cotter Reservoir compared to both other years, sites and historical data, and low abundance of YOY and juveniles was evident in 2012 (see above, Lintermans, unpublished data). Large variability in the number of YOY at the Kissops Flat reference site is of some concern and reduces the likelihood of detecting a statistically significant change in YOY abundance at Cotter Reservoir. A second reference site outside the Cotter Catchment will likely help to clarify localised (site) variation in recruitment for the reference population (the upper Murrumbidgee), which would increase the power to detect change in the level of YOY recruitment in Cotter Reservoir. A second reference site in the upper Murrumbidgee catchment will provide some replication, allowing a better estimate of the status of recruitment of the reference Macquarie perch population.

Conclusion

The current sampling methodology (fyke nets) appears adequate to collect a representative sample of YOY and juvenile Macquarie perch in the ECD and a reference site (Kissops Flat).

Recommendations

2.1 Continue with current sampling methodology for assessing recruitment of YOY Macquarie perch in the ECD to determine if a change occurs as a result of ECD

2.2 Add a second reference site in the upper Murrumbidgee catchment for assessing recruitment of YOY Macquarie perch in the ECD. This will increase the power of detection as currently the reference site is relatively variable in the level of recruitment between years.

3) Will there be significant changes in the abundance, distribution and size composition of adult trout in the ECD?

Trout are a potential major predatory threat to Macquarie perch in the Cotter Reservoir. The enlargement of the reservoir will create a large, thermal refuge for trout (coldwater species) enabling the species to thrive where they are currently limited by high summer water temperatures. Monitoring of changes in the reservoirs trout population will give early warning of potential increases in predatory interactions with Macquarie perch.

Sampling design

To determine the abundance, distribution and size composition of adult trout, Cotter Reservoir and two reference reservoirs (Bendora and Corin Reservoirs) were sampled in autumn each year. Each reservoir was stratified into five sections with two gill nets set per section (in fixed locations) with mesh types randomly allocated each night (Figure 16, Figure 17 & Figure 18). Netting was conducted for five nights at Cotter Reservoir (the focal site) and for two nights at each of the reference reservoirs. Trout were sacrificed and retained for dietary analysis.

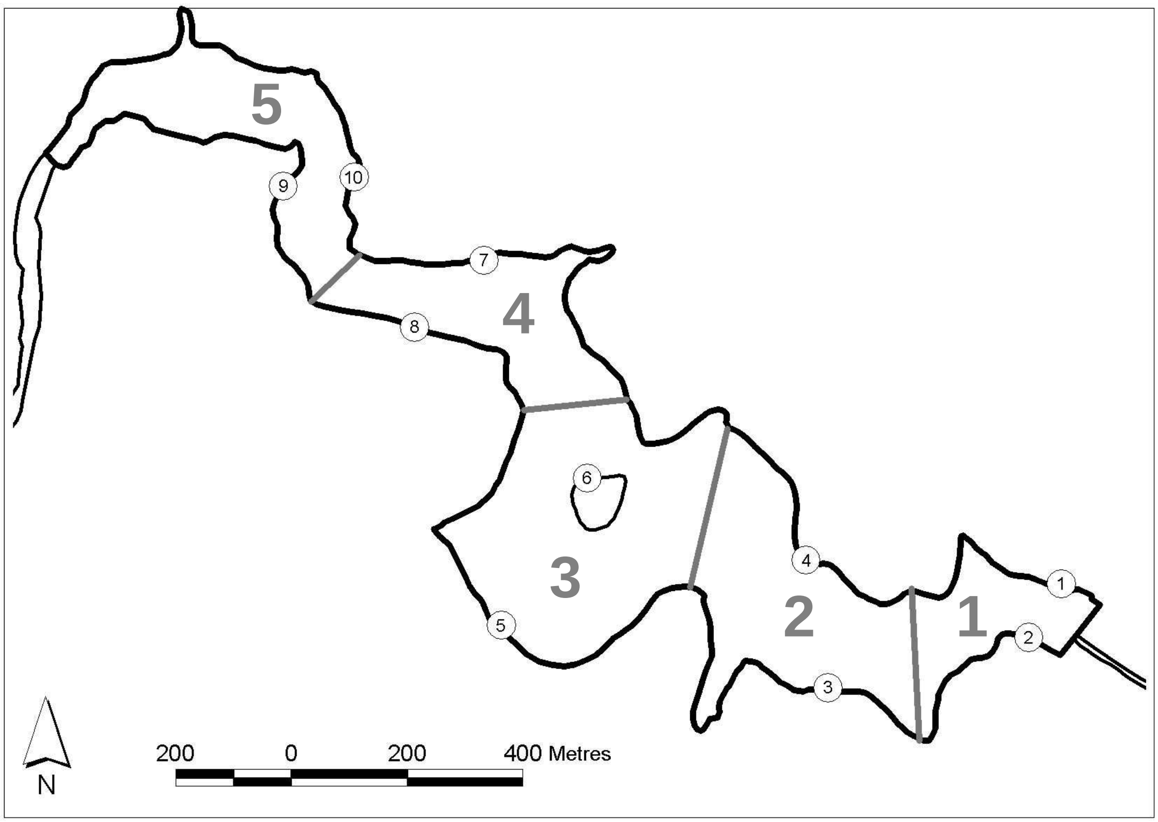


Figure 16. Locations of gill nets (numbers in circles) and sections (marked by grey bars and numbered in grey) in Cotter Reservoir.

Figure 17. Locations of gill nets (numbers in circles) and sections (marked by grey bars and numbered in grey) in Bendora Reservoir.

Figure 18. Locations of gill nets (numbers in circles) and sections (marked by grey bars and numbered in grey) in Corin Reservoir.

Gill nets were set over five nights in autumn 2010 (27/04/2010, 29/04/2010, 04/05/2010, 05/05/2010 & 06/05/2010), 2011 (28/03/2011, 29/03/2011, 04/04/2011, 05/04/2011 & 06/04/2011) and 2012 (02/04/2012, 03/04/2012, 04/04/2012, 02/05/2012 & 03/05/2012) in Cotter Reservoir.

Results

A total of 65 Rainbow trout were caught in Cotter Reservoir 2010 that ranged in size from 215 – 461 mm fork length (FL) (Figure 19). A total of 18 Rainbow trout were caught in autumn 2011 in Cotter Reservoir that ranged in size from 174 – 450 mm fork length (FL) (Figure 20). A total of 39 Rainbow trout and one Brown trout were caught in autumn 2012 in Cotter Reservoir. The Rainbow trout ranged in size from 114 – 500 mm (Figure 21) and the single Brown trout measured 398 mm (FL).

The catch per unit effort of adult trout from Cotter Reservoir was highest in 2010 compared to 2011 and 2012, however the CPUE was higher in 2012 compared to 2011 (Figure 22).
In 2010, CPUE increased with distance from the Cotter Dam wall (i.e. CPUE higher in the most upstream sections) (Table 8). In 2011, there was no trend in the CPUE by section for trout, though section five (the most upstream section) had more than a threefold higher catch rate of Rainbow trout than the other sections (Table 8). In 2012, there was no trend in CPUE by section for trout, however section five (the most upstream section) had the highest CPUE as was the case in 2010 and 2011(Table 8).

Table 8. Mean catch per unit effort (CPUE) (±SE) and mean length (mm FL ± SE) per section of trout in Cotter Reservoir. CPUE is no. of fish per gill-net hour.

	2010		2011		2012	
	CPUE	Length	CPUE	Length	CPUE	Length
Section 1	0.09 ± 0.04	401 ± 22	0.03 ± 0.02	409 ±12	0.06 ± 0.04	268 ± 52
Section 2	0.14 ± 0.05	301 ± 16	0.04 ± 0.03	377 ± 22	0.16 ± 0.05	362 ± 36
Section 3	0.19 ± 0.09	355 ± 17	0.03 ± 0.02	209 ± 35	0.08 ± 0.04	404 ± 9
Section 4	0.20 ± 0.09	337 ± 14	0.03 ± 0.02	395 ± 55	0.07 ± 0.04	358 ± 26
Section 5	0.28 ± 0.12	369 ± 14	0.14 ± 0.04	386 ± 21	0.19 ± 0.10	364 ± 28
Total	**0.18 ± 0.04**	**352 ± 8**	**0.05 ± 0.01**	**368 ± 18**	**0.11 ± 0.03**	**358 ± 16**

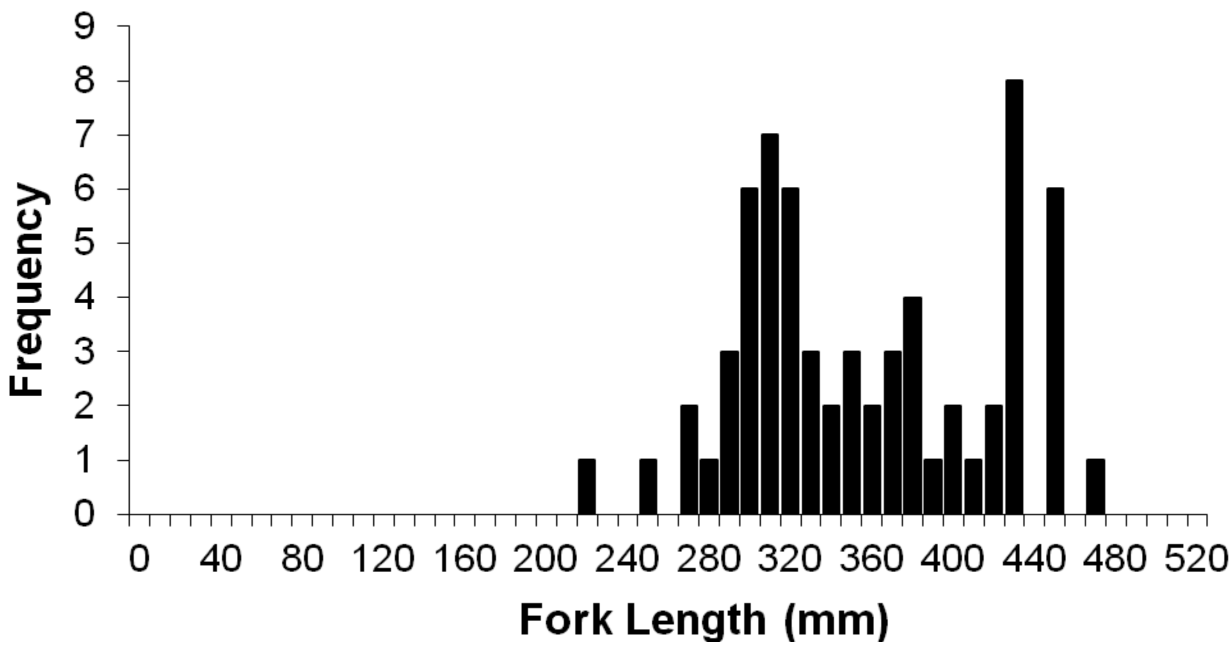


Figure 19. Length of Rainbow trout caught by gill nets from Cotter Reservoir over five nights sampling combined in autumn 2010.

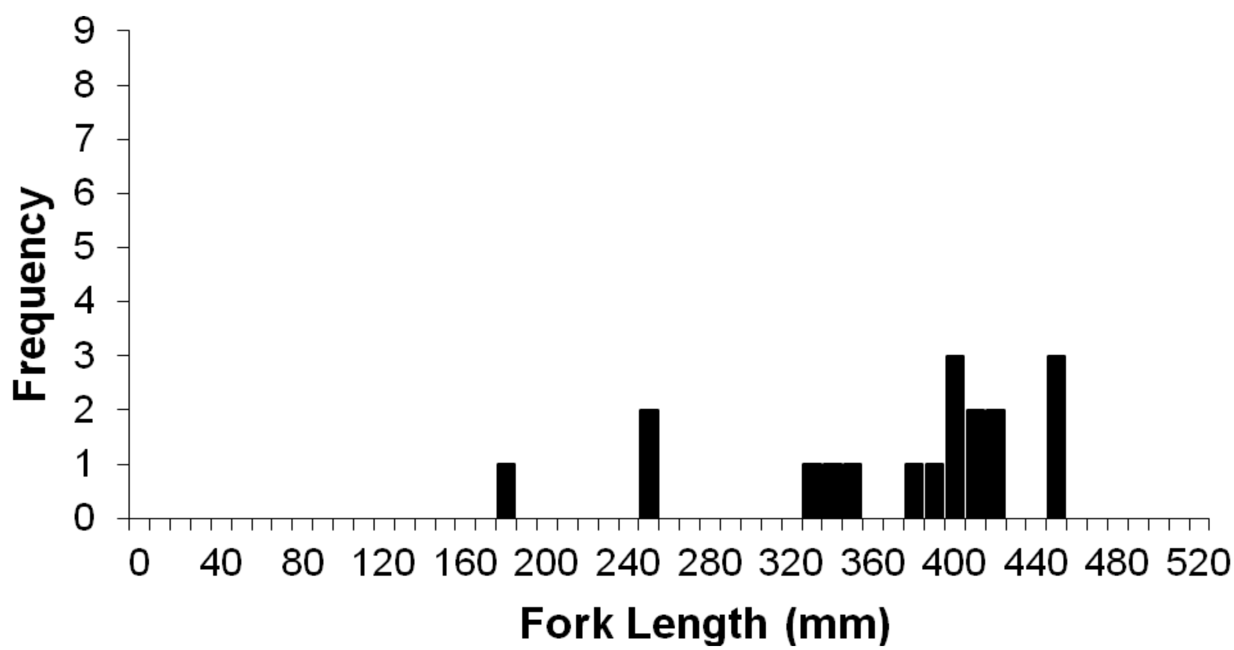


Figure 20. Length of Rainbow trout caught by gill nets from Cotter Reservoir over five nights sampling combined in autumn 2011.

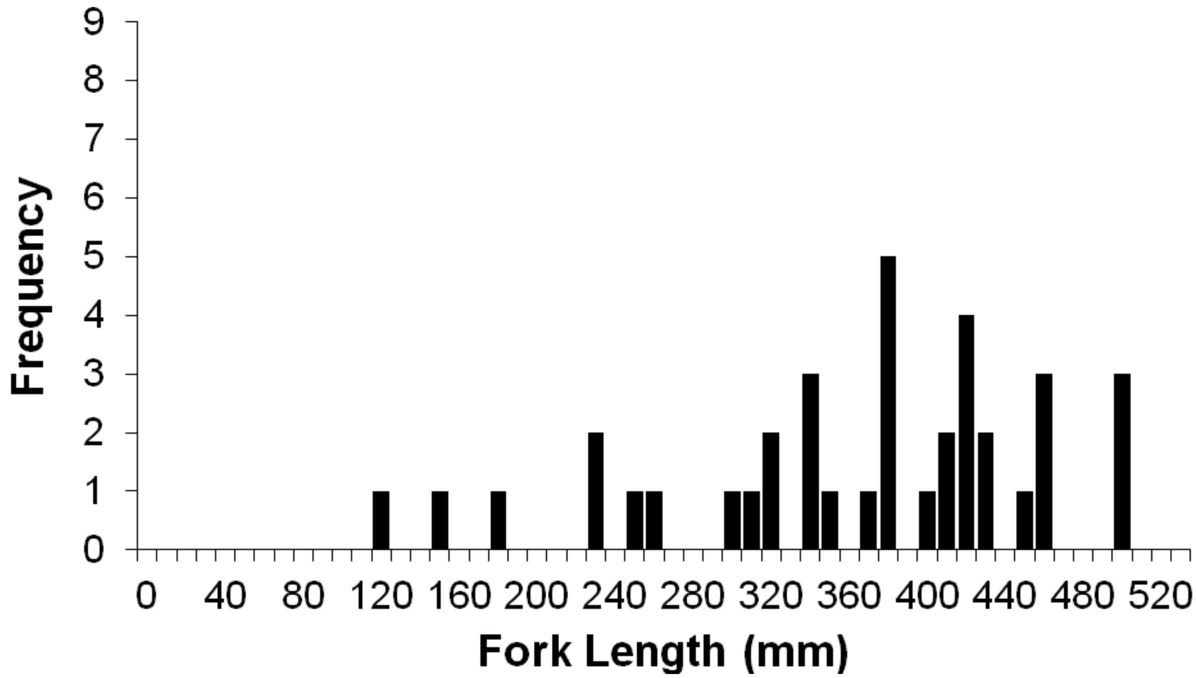


Figure 21. Length of Rainbow trout caught by gill nets from Cotter Reservoir over five nights sampling combined in autumn 2012.

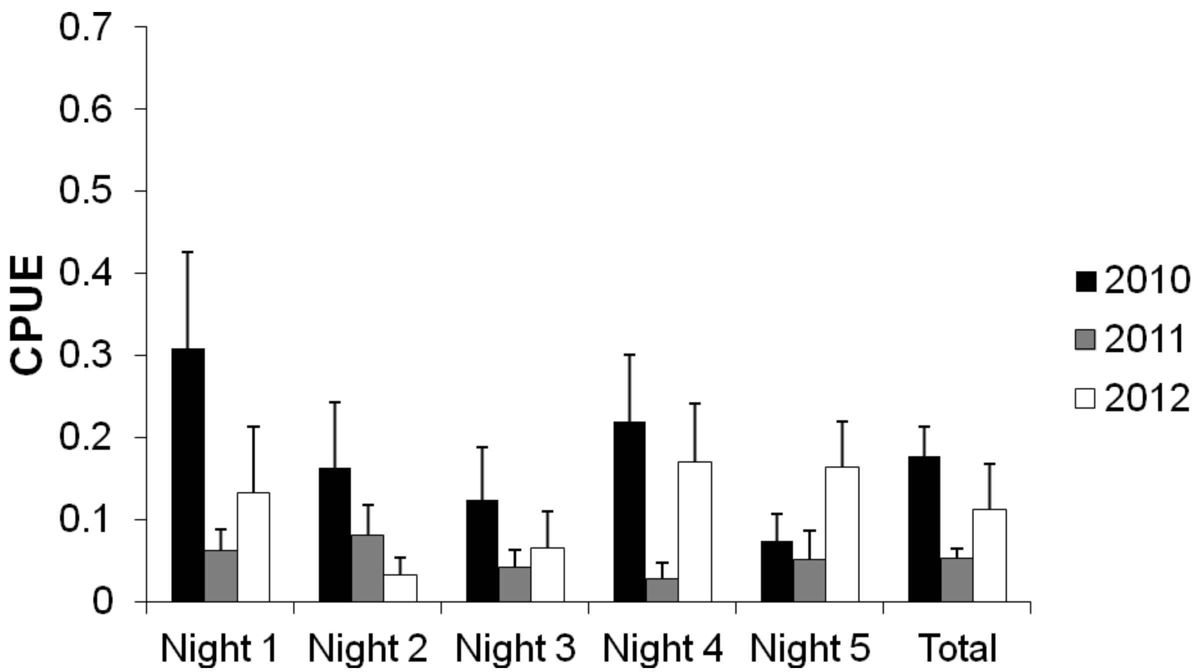


Figure 22. Mean (± SE) Rainbow trout captured per gill-net hour (CPUE) from Cotter Reservoir for each sampling night and total.

Gill nets were set over two nights in autumn 2010 (20/05/201, 27/05/2010), 2011 (09/05/2011 and 11/05/2011) and 2012 (19/04/2012 and 30/04/2012) in Bendora Reservoir. A total of 28 Rainbow trout were caught in 2010 that ranged in size from 279 – 407 mm fork length (FL) (Figure 23).

A total of 44 Rainbow trout were caught in autumn 2011 in Bendora Reservoir that ranged in size from 230 – 407 mm fork length (FL) (Figure 24). A total of 33 Rainbow trout were caught in autumn 2012 (19/04/2012 and 30/04/2012) in Bendora Reservoir that ranged in size from 300 – 375 mm FL (Figure 25) Not that Brown trout do not occur upstream of the Bendora dam wall (Lintermans 2005a).

Capture rates of adult trout in Bendora Reservoir were marginally higher in 2011 than in 2010 and 2012, mostly driven by a high catch rate on night one of sampling (Figure 26). In 2010 catches of Rainbow trout were similar for each section (Table 9). In 2011, Sections three and four had higher catch rates of Rainbow trout compared to other sections (and the same sections in 2010) (Table 9). In 2012, sections four,three and one had the higher catch rates of Rainbow trout compared to the other two sections (Table 9).

Table 9. Mean catch per unit effort (CPUE) (± SE) and mean length (mm FL ± SE) of Rainbow trout per section in Bendora Reservoir. Section 1 is closest to the dam wall. CPUE is no. of fish per gill-net hour.

	2010		2011		2012	
	CPUE	Length	CPUE	Length	CPUE	Length
Section 1	0.28 ± 0.18	336 ± 10	0.17± 0.17	330 ± 13	0.28 ± 0.14	362 ± 8
Section 2	0.15 ± 0.11	316 ± 15	0.20 ± 0.15	343 ± 19	0.04 ± 0.04	366
Section 3	0.27 ± 0.07	334 ± 12	0.41± 0.24	323 ± 12	0.33 ± 0.25	345 ± 7
Section 4	0.19 ± 0.19	332 ± 18	0.75 ± 0.42	339 ± 6	0.50 ± 0.27	344 ± 4
Section 5	0.19 ± 0.04	341 ± 24	0.13 ± 0.13	321 ± 6	0.07 ± 0.04	361 ± 11
Total	**0.22 ± 0.05**	**333 ± 7**	**0.34 ± 0.11**	**333 ± 5**	**0.24 ± 0.08**	**344 ± 3**

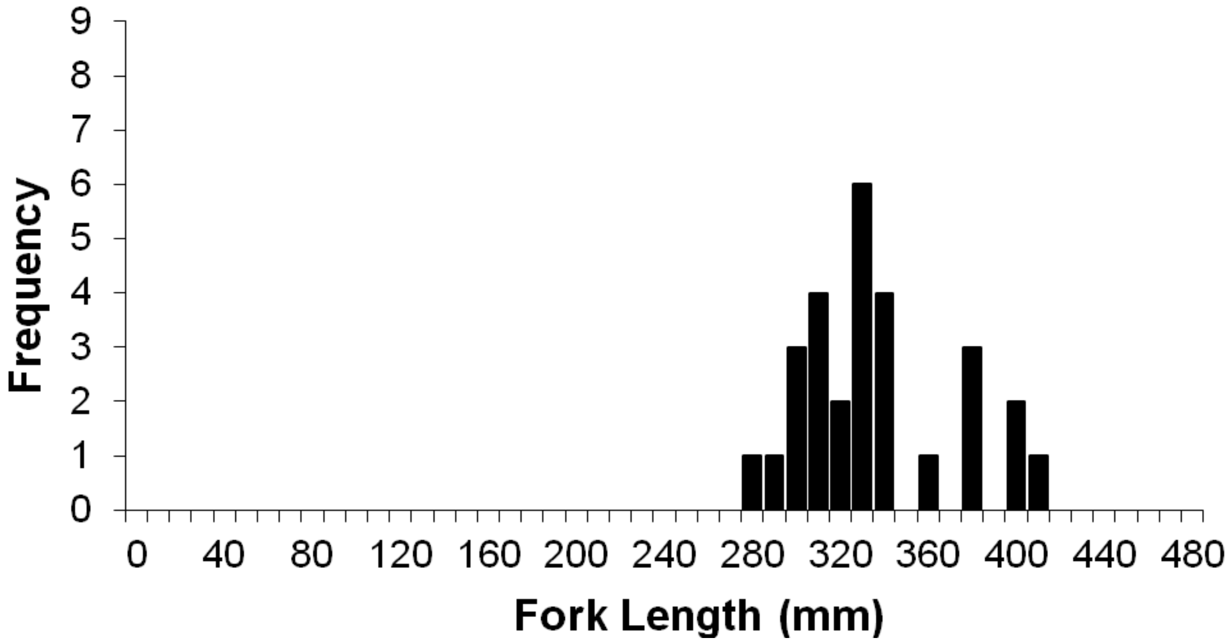


Figure 23. Length of Rainbow trout caught by gill nets from Bendora Reservoir over two nights sampling combined in autumn 2010.

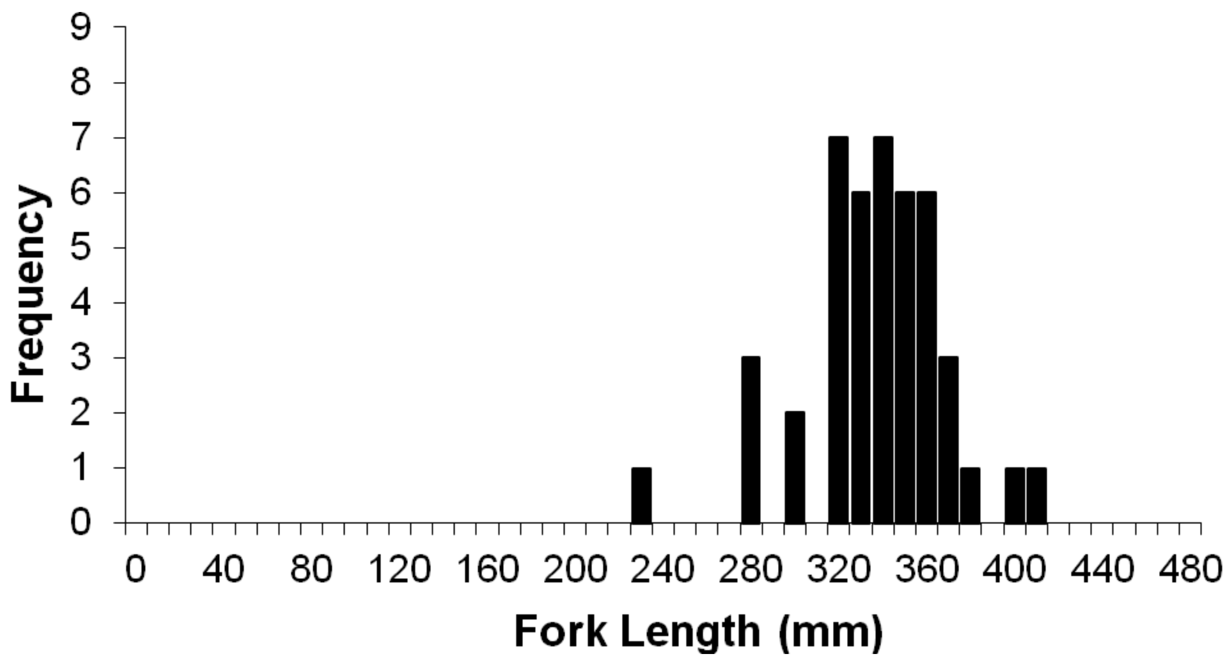


Figure 24. Length of Rainbow trout caught by gill nets from Bendora Reservoir over two nights sampling combined in autumn 2011.

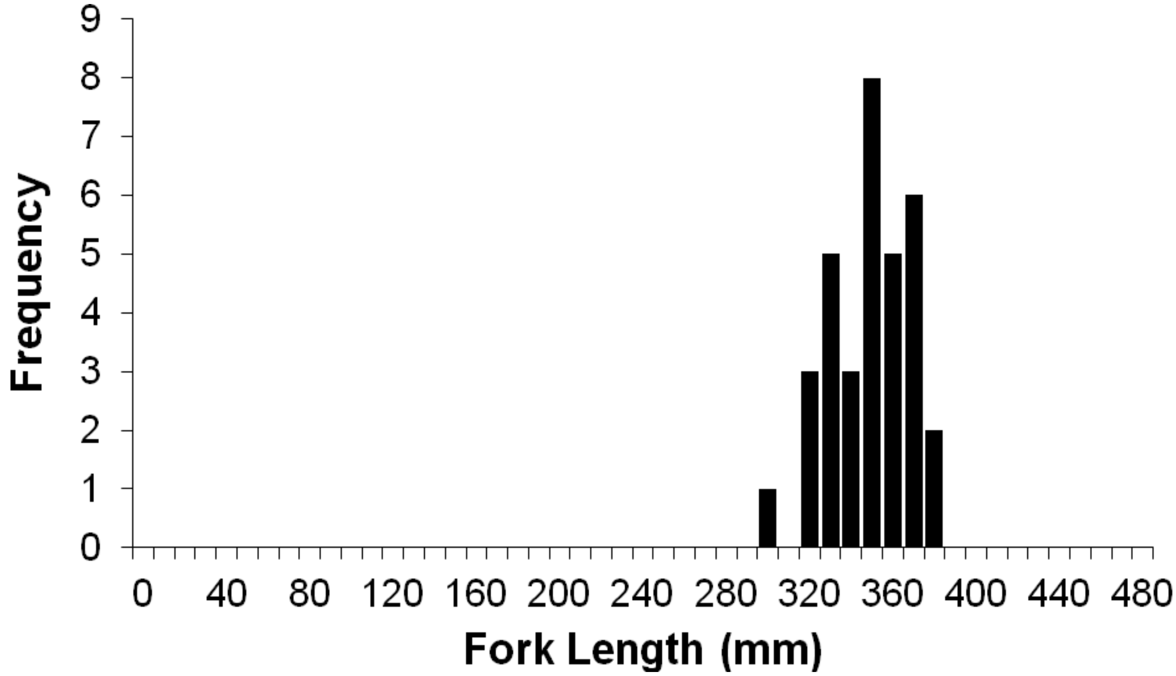


Figure 25. Length of Rainbow trout caught by gill nets from Bendora Reservoir over two nights sampling combined in autumn 2012.

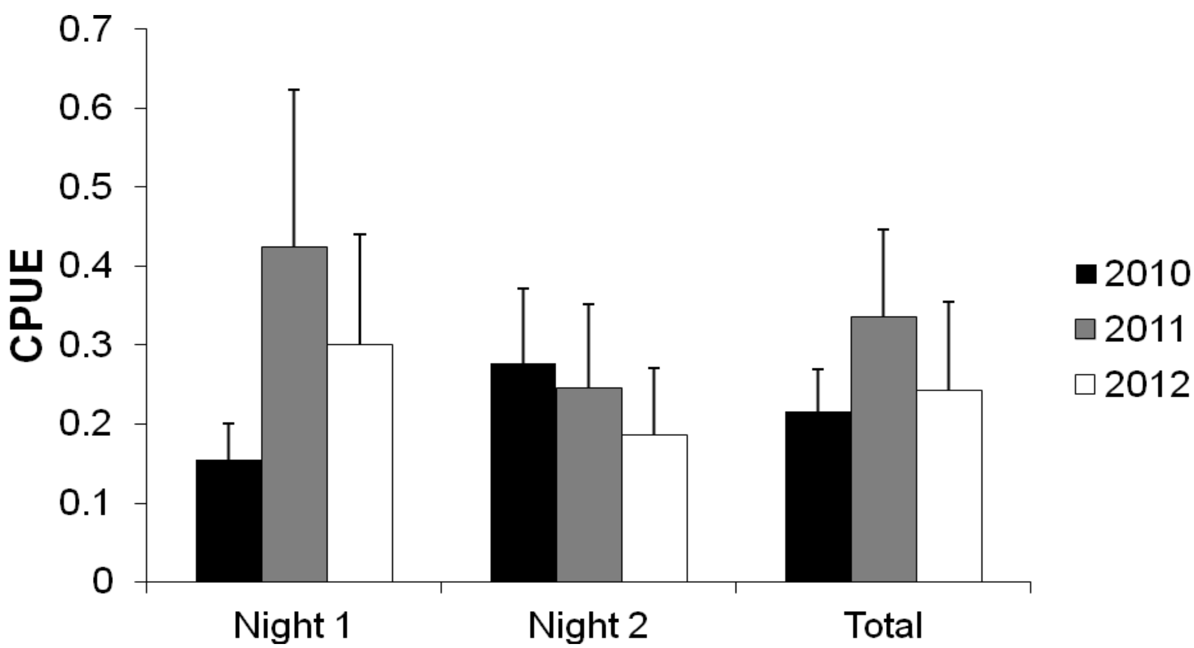


Figure 26. Mean (± SE) Rainbow trout captured per gill-net hour (CPUE) from Bendora Reservoir for each sampling night and total.

Gill nets were set in autumn/winter 2010 (25/05/2010 and 03/06/2010), autumn 2011 (12/05/2011 and 16/05/2011) and autumn 2012 (12/04/2012 and 26/04/2012) in Corin Reservoir. A total of 40 Rainbow trout were caught in 2010 that ranged in size from 240 – 445 mm fork length (FL) (Figure 27). A total of 33 Rainbow trout were caught in autumn 2011 that ranged in size from 256 – 422 mm fork length (FL) (Figure 28). A total of 33 trout were caught in autumn 2012 in Corin Reservoir that ranged in size from 237 – 415 mm FL (Figure 29). Note that brown trout are not present in Corin Reservoir (Lintermans 2000).

Overall catch rates of adult trout in Corin Reservoir were similar in all three years (Figure 30). Catches of Rainbow trout were relatively consistent between sections and years, with the exceptions of sections two and four in 2010, and sections two and five in 2011 (Table 10). Section two in 2010 had higher catches of trout compared to other sections both within and among years (Table 10). Sections four (2010), two and five (2011) had low catches of rainbow trout within and among sections and years (Table 10). In 2012, there was no trend in CPUE per section for Rainbow trout (Table 10). Section four had the highest CPUE which was almost double the catch rate for the nearest section (section 2) (Table 10).

Table 10.Catch per unit effort (CPUE) (± SE) and mean length (mm FL ± SE) per section of Rainbow Trout in Corin Reservoir. Section 1 is closest to the dam wall. CPUE is no. of fish per gill-net hour

	2010		2011		2012	
	CPUE	Length	CPUE	Length	CPUE	Length
Section 1	0.27± 0.27	362 ± 10	0.31 ± 0.27	346 ± 13	0.11 ± 0.11	368 ± 19
Section 2	0.49 ± 0.24	328 ± 15	0.15 ± 0.10	297 ± 41	0.26 ± 0.15	344 ± 17
Section 3	0.25 ± 0.06	303 ± 16	0.31 ± 0.27	334 ± 5	0.08 ± 0.05	320 ± 15
Section 4	0.14 ± 0.09	374 ± 24	0.36 ± 0.22	325 ± 7	0.50 ± 0.20	350 ± 9
Section 5	0.27 ± 0.22	332 ± 15	0.10 ± 0.06	409 ± 11	0.24 ± 0.14	365 ± 6
Total	**0.28 ± 0.08**	**336 ± 8**	**0.25 ± 0.08**	**340 ± 7**	**0.24 ± 0.07**	**351 ± 6**

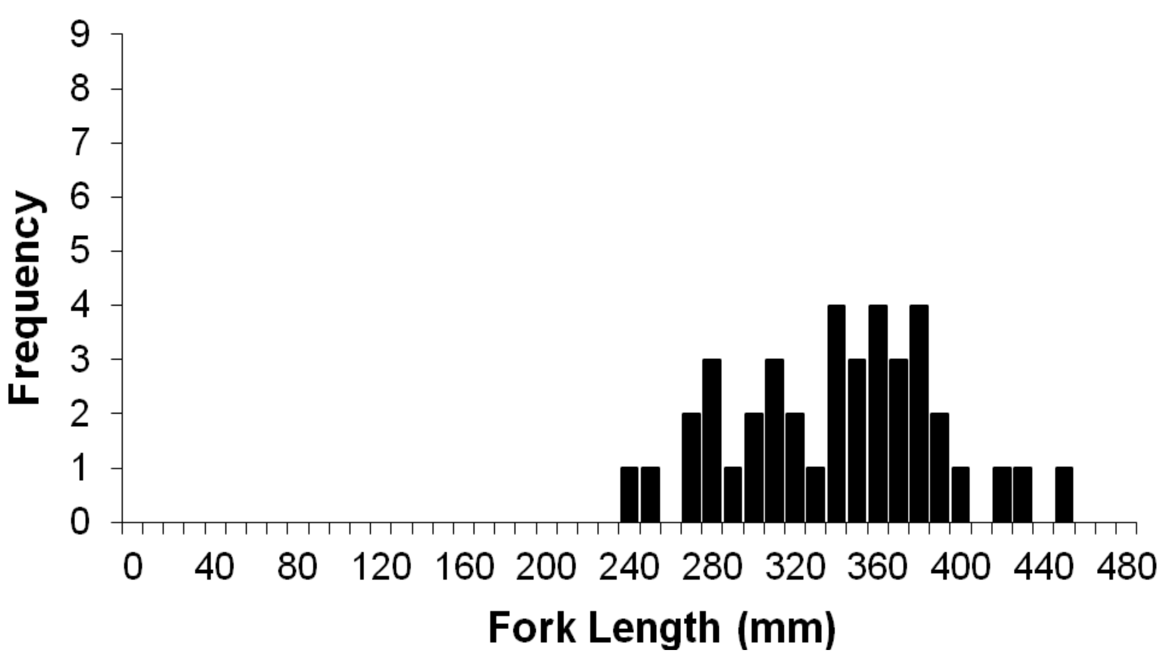


Figure 27. Length of Rainbow trout caught by gill nets from Corin Reservoir over two nights sampling combined in autumn 2010.

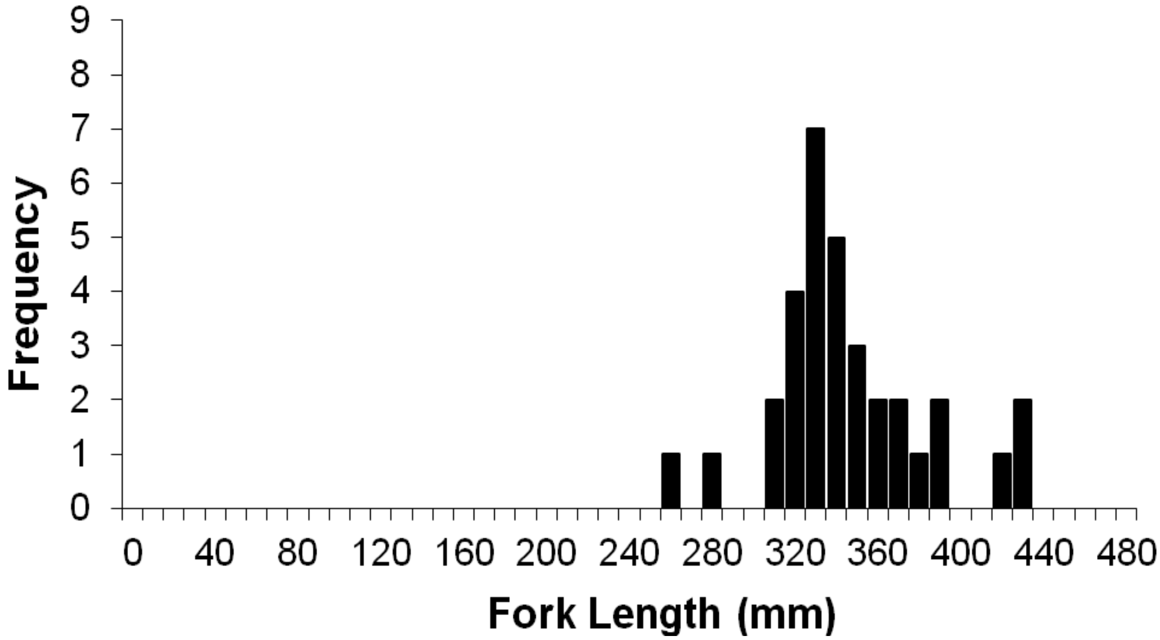


Figure 28. Length of Rainbow trout caught by gill nets from Corin Reservoir over two nights sampling combined in autumn 2011.

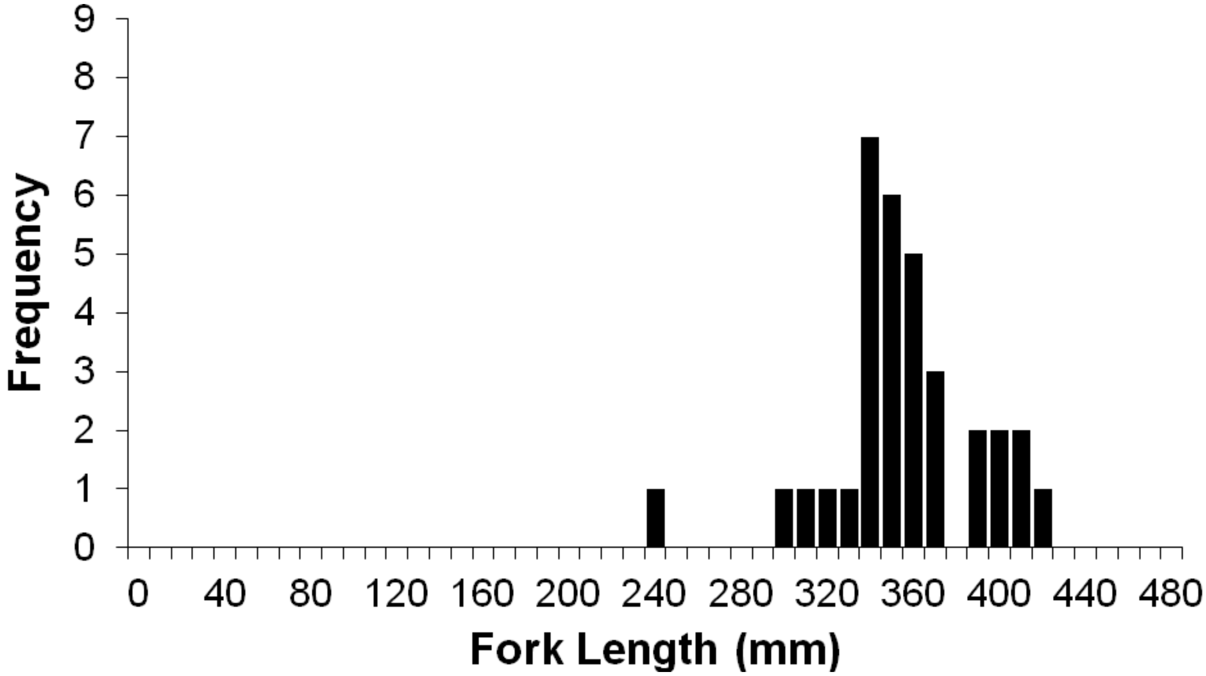


Figure 29. Length of Rainbow trout caught by gill nets from Corin Reservoir over two nights sampling combined in autumn 2012.

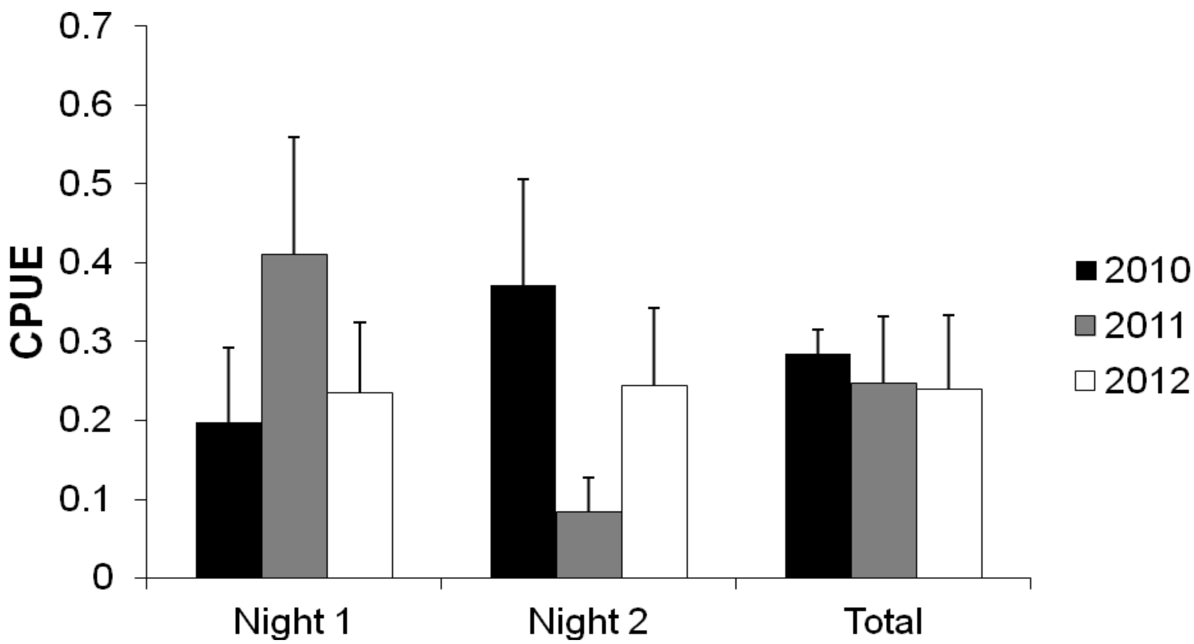


Figure 30. Mean (± SE) Rainbow trout captured per gill-net hour (CPUE) from Corin Reservoir for each sampling night and total.

Discussion

Rainbow trout were a common capture in all three reservoirs, with Corin and Bendora Reservoirs having higher abundance compared to Cotter Reservoir. The low abundance of trout in Cotter Reservoir is likely a result of the prolonged drought, the lower elevation of Cotter Reservoir (hence, higher water temperatures), and high sediment load from the bushfire-affected commercial forestry plantations. However, it is likely that trout abundance will increase in Cotter Reservoir during filling, as thermal refuge habitat and food resources increase (Lintermans 2012). Abundances of Rainbow trout were consistent across years for Corin and Bendora Reservoirs, but Cotter Reservoir exhibited some variability with relatively low capture rate in 2011. The relatively constant abundances observed suggest that the current survey design is adequate for monitoring adult trout abundance.

The three reservoirs had a very similar size composition of adult trout, with most individuals caught measuring between 240 – 400 mm fork length (FL). The sampling regime employed appears to be adequate in capturing a representative sample of adult trout in reservoir environments.

Highest catches of adult trout in Cotter Reservoir were generally from site five (the most upstream and shallow site). This may be caused by a couple of factors: 1) that the gill nets, which hang 2 – 4 m down into the water column, sample a larger proportion of the water column in the shallower water or 2) that abundances of adult trout are at their greatest in the upstream section of the reservoir. Ebner *et al*. (2007) indicated that the trout were more abundant in the upstream third of the Cotter Reservoir when compared to the deeper downstream sections.

Trout captures between sections in Bendora Reservoir did not follow a discernible pattern over the three years, other than generally the highest capture rates coming from the middle sections of the reservoir. Corin Reservoir also did not exhibit a variable pattern in capture rates between sections, with relatively uniform captures rates across most sections across years. These results indicate that trout do not exhibit a discernible preference for their location in Corin and Bendora Reservoirs and that their distribution is relatively uniform across the five sections of each reservoir.

Conclusion

The sampling regime appears to be adequate for detecting change of adult trout numbers in the ECD due to relatively stable levels of trout in the reference reservoirs. The abundance of trout at the upper end of the size spectrum will be of major interest once ECD starts to fill as it is expected that more large trout will be present.

Recommendations

3.1 The current sampling design should remain unchanged for assessing significant changes in the abundance, distribution and size composition of adult trout in the ECD.

4) What are the levels of predation on Macquarie perch larvae and juveniles by trout in the ECD and river upstream?

Predation by trout of Macquarie perch young of year or juveniles has never been documented in the reservoir, with rare occurrences of juvenile predation documented from the river upstream. Therefore, trout predation on juveniles in the reservoir is not considered a major threat. However, trout predation on larval Macquarie perch has never been examined and forms the most pressing element of this monitoring question. Macquarie perch breed later in the year than trout, so predation on Macquarie perch larvae may be occurring from juvenile, sub-adult and adult trout. Larvae are highly digestible and are probably only identifiable in trout stomachs for a few hours at most. Consequently, previous trout sampling where fish have been captured in overnight netting operations have potentially missed evidence of larval predation.

Sampling design

Trout (both rainbow and brown) were collected from six riverine sites between Cotter Reservoir and Bendora Dam (Bracks Hole, Vanitys Crossing, Spur Hole, Pipeline Rd. Crossing, Burkes Ck. Crossing and Downstream of Bendora Dam) by backpack electrofishing. Sampling effort at each site consisted of backpack electrofishing 1km of river, or the collection of 20 trout (>150 mm – size at which trout gape would be sufficient to ingest a juvenile Macquarie perch), whichever occurred first. Sampling was designed to be conducted in late November – early December when Macquarie perch larvae and juveniles are present in the river. Trout stomachs were removed in the field, and contents stored in 100% ethanol to be later sorted under stereo-microscope in the laboratory. Fish and crayfish found in stomach contents were identified to species (where possible), with insects classified as either aquatic or terrestrial based on their origin. Estimates of the composition of dietary items were made based on percent volume of each item of the total volume of all items. Stomach contents were retained to allow future genetic identification of fish remains, particularly of larvae.

Sampling was not able to be undertaken during the target period in 2010 and 2011 as a result of high flows in Cotter River from heavy rainfall.

Results

No Macquarie perch were detected in trout diet in the single year that trout stomachs were collected during the target period (01/11/2012 to 05/12/2012). A total of 37 trout were captured comprising 36 Rainbow trout and one Brown trout (Table 14 and Figure 45). Aquatic invertebrates were the most common food item, present in 100% of stomachs followed by terrestrial invertebrates (81%) and plant material (6%). The Brown trout (500 mm FL) captured had two Rainbow trout in its stomach, measuring 140 and 180 mm FL.

To enable the detection of Macquarie perch DNA in trout stomachs, a specific laboratory diagnostic test needs to be designed and validated. A small project was carried out in 2012 at the Institute of Applied Ecology genetics laboratory the University of Canberra, but lack of sufficient funding meant that further work is still required (see Appendix A). A proposal is currently being prepared to seek funding support external to the ECD Fish Management Program to allow this additional work to be conducted.

Discussion

Heavy rains (and associated high river flows) in spring and early summer in 2010 and 2011 prevented a spring sample of trout diet from being obtained from the Cotter River. An alternative sampling method that can be deployed during high flows (such as angling) would be beneficial. In the one year that a trout diet sample was collected during the target Macquarie perch larval period, no Macquarie perch were detected visually in trout stomachs.

The initial development of a Macquarie perch genetic detection test (Appendix A) demonstrates that the concept of genetic detection of Macquarie perch is feasible, but further work is required to identify the sensitivity of the test, and how effective it is on partially-digested material (amongst other additional refinements required). This additional work will require aquaria experiments with trout to investigate how many larvae would need to be consumed to be genetically detectable, and how long after larval ingestion a positive result is likely.

A proposal is currently being prepared to seek funding support external to the ECD Fish Management Program to allow this additional work to be conducted.

Conclusion

With the current techniques no predation of Macquarie perch has been detected. It is still likely that some level of predation of early juveniles occurs, but visual examination of trout stomach contents is too crude to detect this. Other techniques such as genetic detection would increase the chances of detecting predation of early juvenile Macquarie perch by trout.

Recommendations

4.1 Explore alternative trout collection techniques (angling) to assess predation on Macquarie perch larvae if high flows prevent backpack electrofishing. This will ensure that sampling seasons are not missed as this reduces the power of the sampling design to detect change over the course of seasons and years.

4.2 Further develop genetic detection capabilities to detect Macquarie perch in the diet of trout.

4.3 In addition to the spring trout sample collected for Q7, collect a separate sample of trout to examine potential predation on Macquarie perch. This will spread the risk of environmental conditions preventing both samples from being collected.

5) Will there be significant changes in the abundance, distribution and species composition of piscivorous birds in the ECD?

Piscivorous birds (predominantly cormorants) have been identified as a potential threat to Macquarie perch in the ECD. Predation of Macquarie perch by cormorants in Cotter Reservoir has been confirmed and a significant expansion of the piscivorous bird population following enlargement of the reservoir could have severe consequences on the small adult population size of Macquarie perch. Assessment of population trend in piscivorous birds on Cotter Reservoir is required.

Sampling design

Surveys of the location and abundance of piscivorous birds on Cotter Reservoir were conducted monthly. The species counted were: Great cormorants, Little black cormorants, Little pied cormorants, and Pied cormorants. Surveys were conducted using 10 x 42 mm binoculars from a boat with the number and location of individuals recorded on a map. For the purposes of this question, Cotter Reservoir was divided into 5 sections (the same sections used for gill netting – Figure 16), then the location of counted individuals assigned to the section in which it was observed. Counts were be made in the morning (0900 – 1100 hrs) whenever possible.

Results

A total of 689 cormorant observations from three species were recorded over 26 surveys from 9/7/2010 to 28/12/2012 at Cotter Reservoir. The most numerous species were Great cormorant (367) followed by Little pied cormorant (208) and then Little black cormorant (114). The maximum number observed during any one counting day was 67 for Great cormorant (28/11/2012), 44 for Little pied cormorant (11/11/2011) and 16 for Little black cormorant (11/11/2011) (Figure 31). Great cormorant was more abundant during the warmer months and its numbers increased across years (Figure 31). Apart from a spike in abundance in summer 2011/2012 Little pied cormorant abundance was relatively stable throughout the study (Figure 31). Little black cormorant abundance was relatively stable over the study period (Figure 31).

Great cormorant, Little black cormorant and Little pied cormorant were most abundant at the upstream end of the Cotter Reservoir (section five) with 183 (50%), 45 (39%) and 99 (48%) observations of each species recorded there, respectively (Figure 32).

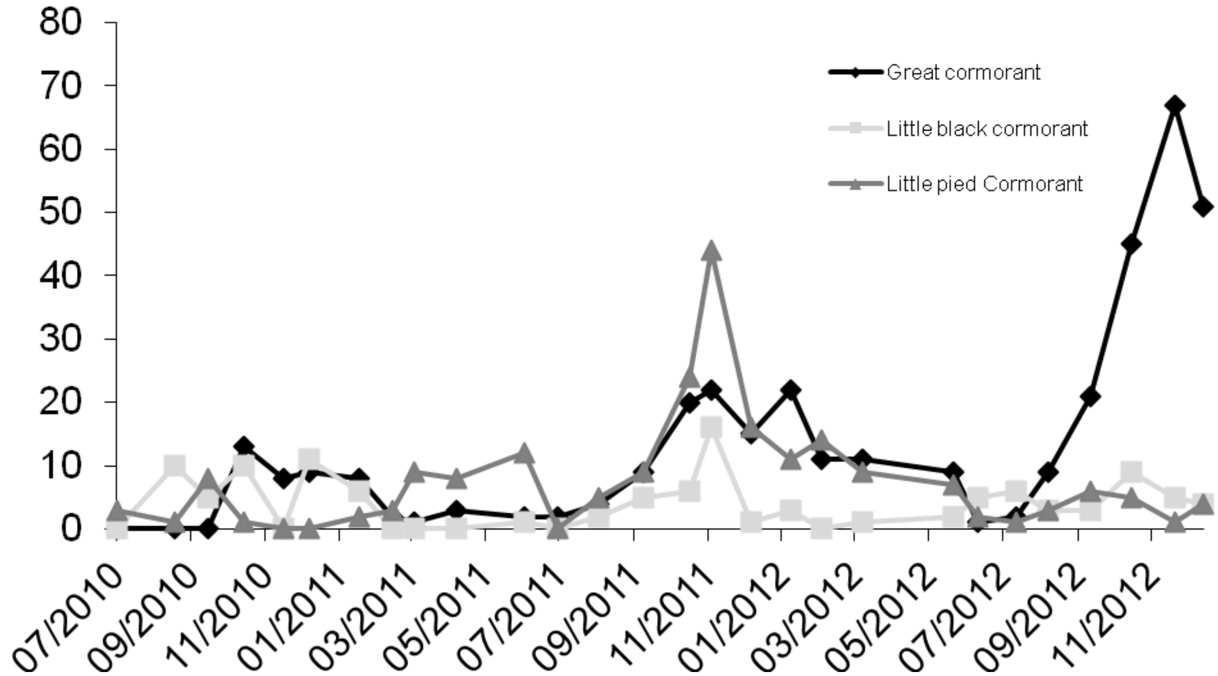


Figure 31. Number of cormorants of each species (black line = Great cormorant, light grey line = Little black cormorant and Dark grey line = Little pied cormorant) observed during each monthly survey of Cotter Reservoir.

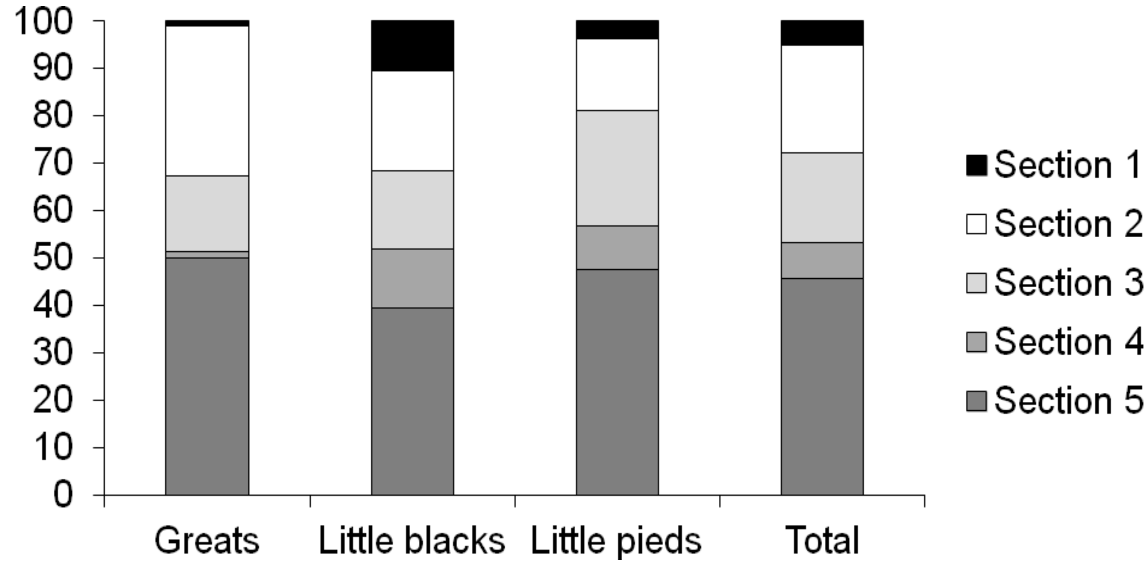


Figure 32. Percentage of individuals observed in each section of Cotter Reservoir between July 2010 and December 2012. Total is all species combined.

Discussion

In general cormorant abundances (all three species) were lowest during the cooler months of the year (June – August). A spike in the abundance of all three species occurred in spring 2011, especially Little pied cormorants which increased to a peak of four-fold their normal abundances. Great cormorant abundances were relatively high (four-fold normal abundances) during spring of 2012, though the other two species remained at low level abundances. The reason for the spike in Great cormorant abundance in 2012 is unknown, but may be related to the temporary flooding that occurred in early 2012. These trends of generally more piscivorous birds being present in warmer months were also observed by Ryan (2010). The increase in cormorant abundance in warmer months corresponds with the spawning and nursery period of many native and alien fish species present in Cotter River including Macquarie perch, Goldfish, and Eastern gambusia (Lintermans 2007), as well as crustaceans such as Yabby and Freshwater prawn (Williams 1977). Freshwater prawn, Macquarie perch and Goldfish were found to be important dietary items of cormorants in Cotter Reservoir (Lintermans *et al.* 2011). The increase in water temperatures and the associated spawning and nursing timing of fish and crustaceans are most likely the drivers for the general increase in cormorant numbers during warmer months.

All cormorant species were most commonly found at the upstream end of the Cotter Reservoir. The upstream 5th of the reservoir (section five in the current study) is still relative wide (~100 m) but is shallow, rarely exceeding < 5 m in depth, and is mostly < 3 m depth (Ryan 2010). Previous research has found that cormorants commonly hunt in depths of less than 5 m (Dorfman and Kingsford 2001; Ropert-Coudert *et al.* 2006), though have been recorded diving to depths greater than 30 m (Kato *et al.* 2006). The upstream area of the Cotter Reservoir is also the location of aggregations of Macquarie perch in September – November as adults of this species prepare to move into the river to spawn (Ebner and Lintermans 2007; Lintermans *et al.* 2010; Ryan 2010).

Conclusion

The visual survey technique employed in this study appears to be relatively effective in gauging the relative abundance of Cormorant species. Results of monthly survey are to be incorporated in the Cormorant Management Plan.

Recommendation

5.1 The current sampling design should remain unchanged for assessing abundance, distribution and species composition of piscivorous birds in the ECD.

6) Will there be significant changes in the abundance and size composition of trout in the Cotter River upstream of the ECD?

As the trout population in the ECD is likely to increase as a result of expanded habitat availability and increased access to thermal refugia, it is probable that there will be an increase in trout abundance in the river upstream of the ECD. This is because trout must enter the river to spawn in flowing waters. Monitoring of changes in trout abundance and size distribution in the river will provide insight into potential increases in predatory or competitive interactions with threatened fish. Seasonal monitoring is required as all Blackfish size classes are small enough to be prey (i.e. different to Macquarie perch when larvae are only available in late spring/early summer).

Sampling design

Six riverine sites were sampled between Cotter Reservoir and Bendora Reservoir, these were (in order from most downstream to most upstream); Bracks Hole, Vanitys Crossing, Spur Hole, Pipeline Road. Crossing, Burkes Creek. Crossing and Downstream of Bendora Dam. Reference sites within the Cotter River catchment (Cotter Hut) and outside the Cotter River catchment (Micalong Creek), was also sampled. Sampling was conducted each year between summer and early autumn. Fyke nets and back pack electrofishing were employed to catch trout at each site. At each site, three fyke nets were set in each of four pools and four 30 m sections of river were sampled by backpack electrofishing. Vanitys Crossing has only one large pool, so all 12 fyke nets were set in the one pool.

Sampling was conducted in summer 2010 (8/2/2010 – 22/2/2010), autumn 2011 (07/03/2011 – 07/04/2011) and summer 2012 (18/01/2012 – 09/02/2012).

Results

In 2010 a total of 71 Rainbow trout were captured from all sites combined ranging in size from 76 – 332 mm FL (Figure 33).
A total of 24 Rainbow trout were caught in 2011 ranging in size from 79 – 285 mm FL (Figure 34). In 2012 a total of 40 Rainbow trout were captured from all sites combined ranging in size from 73 – 316 mm FL (Figure 35). A total of three Brown trout were also captured in 2012 ranging in size from 220 – 265 mm FL. In 2010, 2011 and 2012 catches of trout species increased with distance upstream from Cotter Reservoir, and the site immediately downstream of Bendora Dam had the greatest catch of trout (

Table 11). With the exception of Cotter Hut (which recorded a small increase in CPUE), catches of Rainbow trout were lower in 2011 than 2010 and 2012 (

Table 11).

Table 11. Number and mean length (mm FL ± SE) of Rainbow trout caught in fyke nets and by backpack electrofishing in Cotter River in 2010, 2011 and 2012.

	2010		2011		2012	
	n	Length	n	Length	n	Length
Bracks Hole	0	n/a	0	n/a	2	86 ± 6
Vanitys Crossing	0	n/a	0	n/a	0	n/a
Spur Hole	3	94 ± 7	1	160	5	140 ± 33
Pipeline Rd. Crossing	6	174 ± 40	4	159 ± 30	6	225 ± 43
Burkes Ck. Crossing	6	172 ± 33	2	242 ± 20	7	210 ± 30
D/S Bendora	56	124 ± 6	17	198 ± 17	20	121 ± 16
Cotter Hut	12	134 ± 15	13	209 ± 14	7	177 ± 36

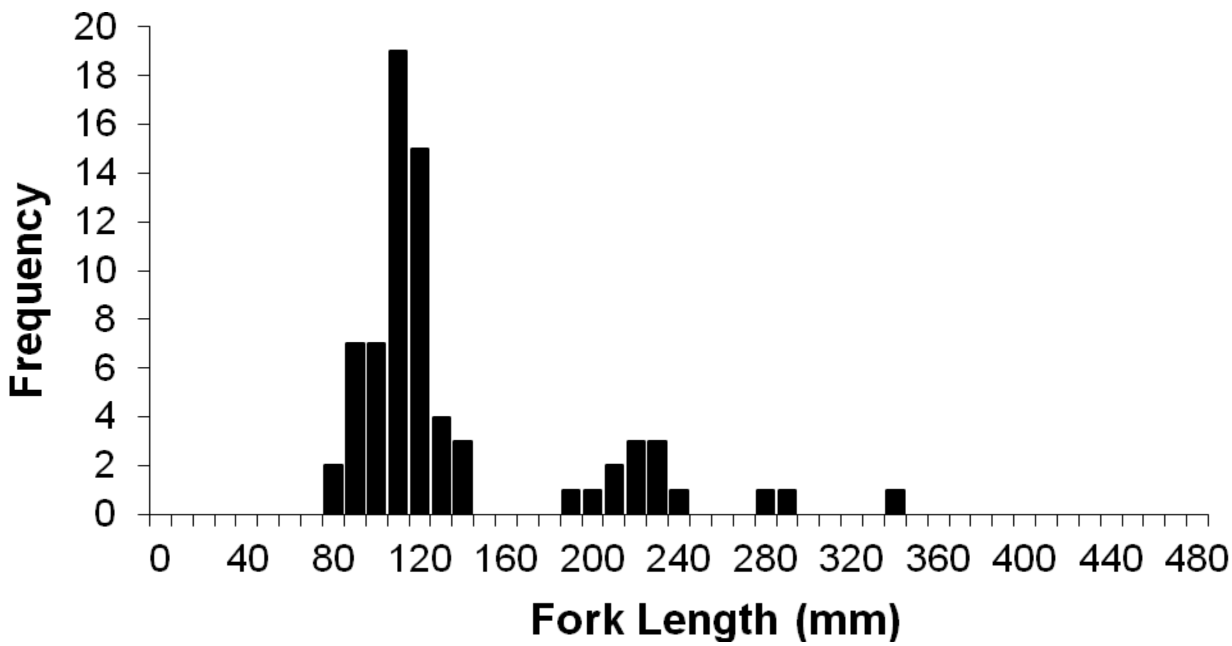


Figure 33. Length of Rainbow trout caught by two methods (fyke nets and electrofishing) from six sites between Cotter and Bendora Reservoirs in February 2010.

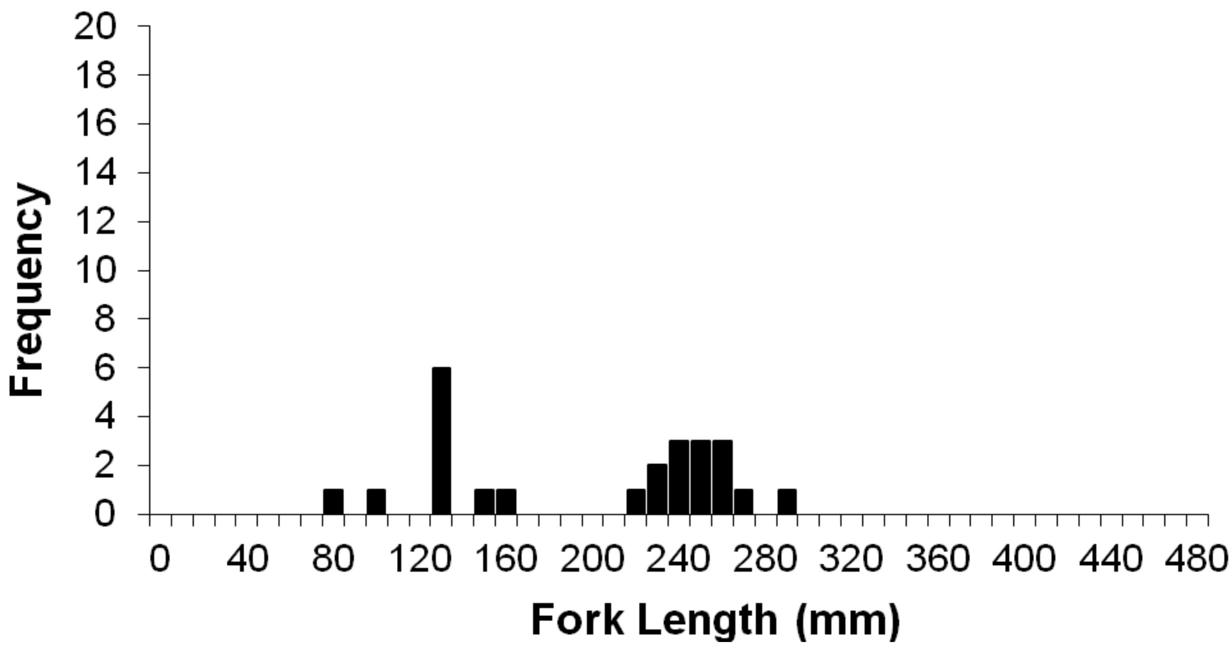


Figure 34. Length of Rainbow trout caught by two methods (fyke nets and electrofishing) from six sites between Cotter and Bendora Reservoirs in March/April 2011.

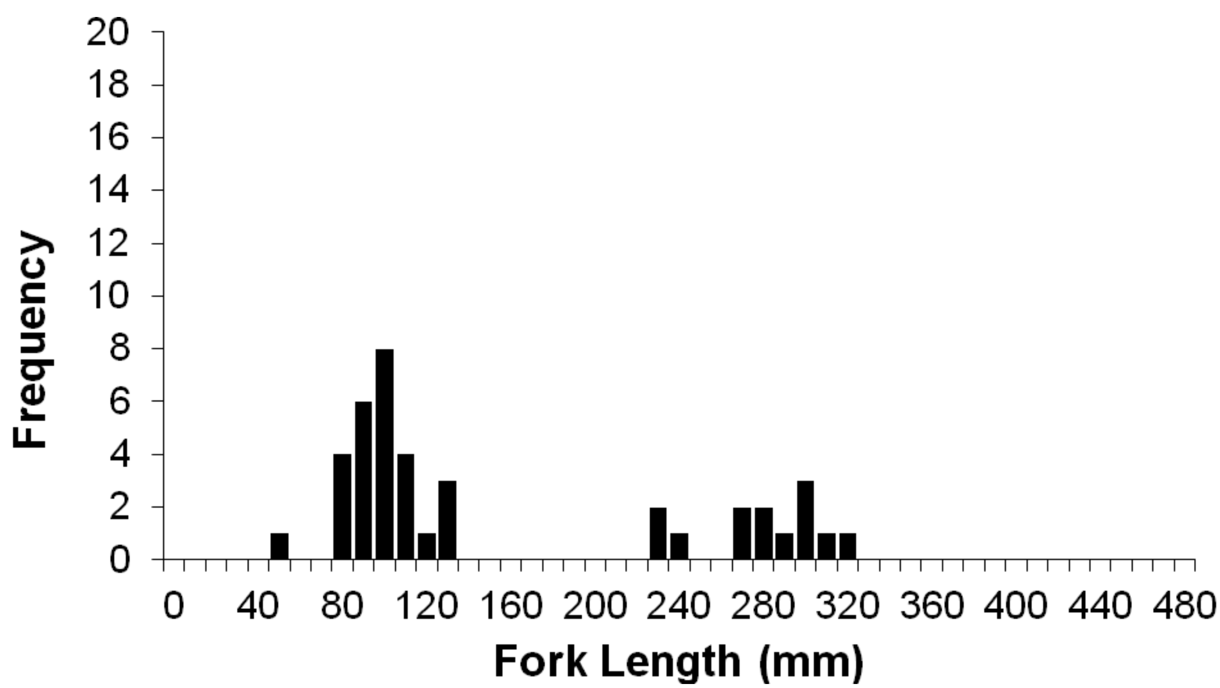


Figure 35. Length of Rainbow trout caught by two methods (fyke nets and electrofishing) from six sites between Cotter and Bendora Reservoirs in summer 2012.

A reference site, Cotter Hut, above Corin Reservoir was also sampled in summer 2010 (18/02/2010), autumn 2011 (07/04/2011 and 08/04/2011) and summer 2012 (24/01/2012). A total of 12 Rainbow trout were caught in 2010 ranging in size from70 – 237 mm FL (Figure 36) and a total of 13 Rainbow trout caught in 2011 (117 – 291 mm FL (Figure 37)) and a total of seven Rainbow trout captured in 2012 (54 – 304 mm FL (Figure 38)).

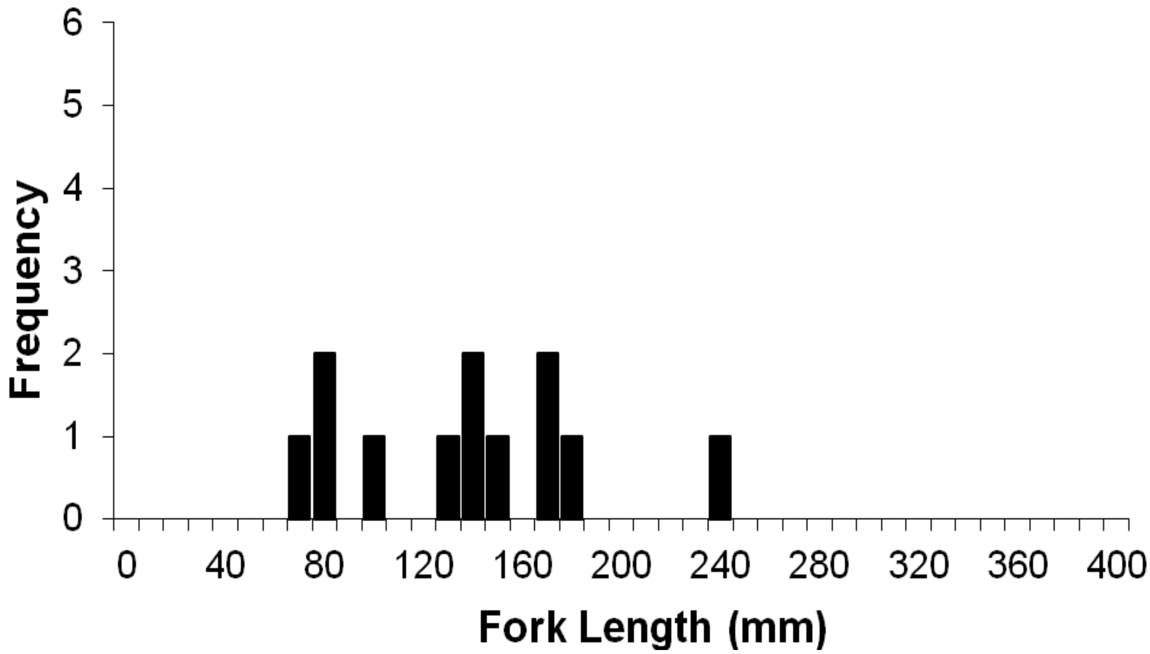


Figure 36. Length of Rainbow trout caught by two methods (fyke nets and electrofishing) from Cotter Hut in February 2010.

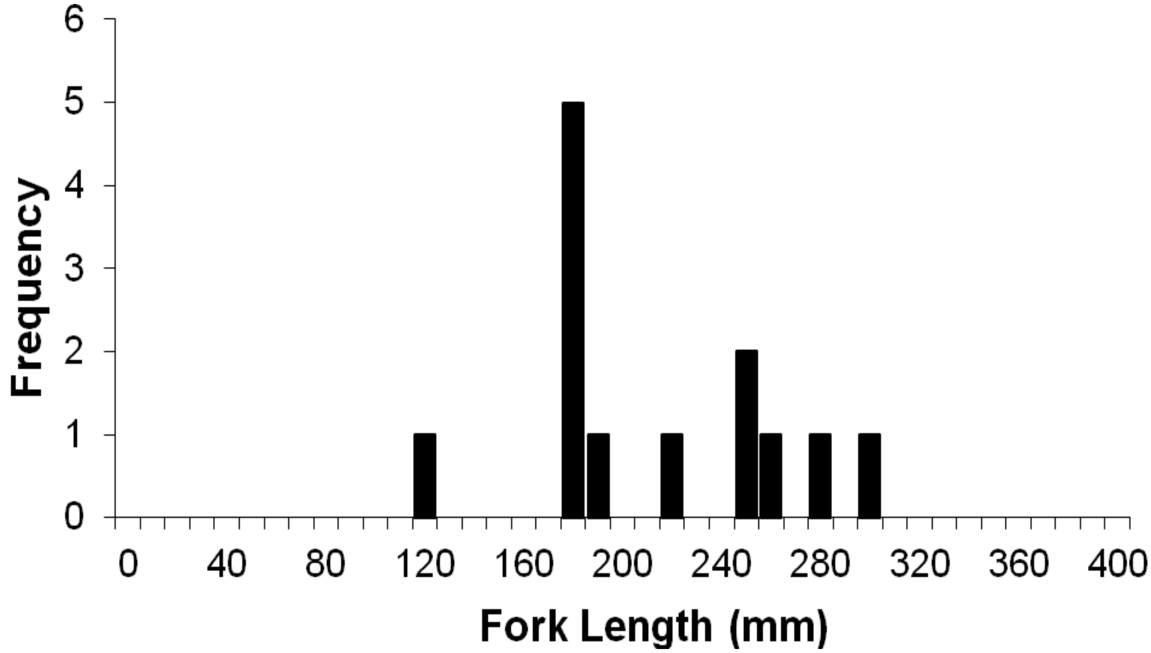


Figure 37. Length of Rainbow trout caught by two methods (fyke nets and electrofishing) from Cotter Hut in April 2011.

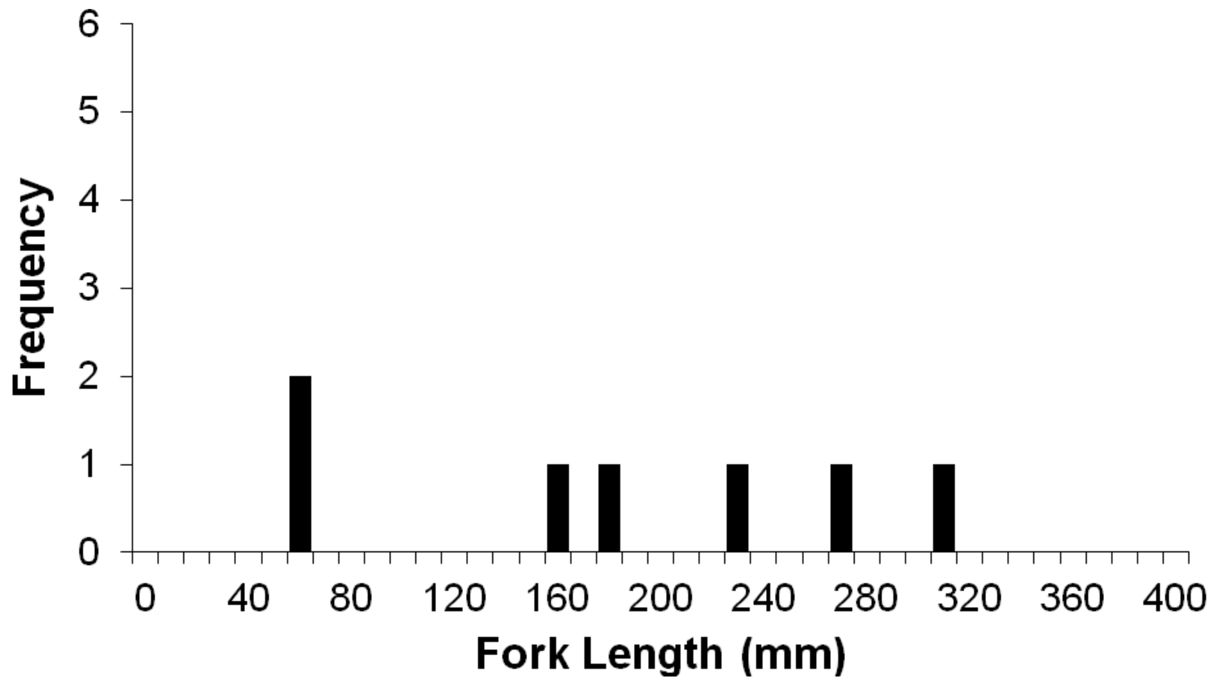


Figure 38. Length of Rainbow trout caught by two methods (fyke nets and electrofishing) from Cotter Hut in January 2012.

An external reference site to the Cotter catchment, Micalong Creek, (Goodradigbee River catchment) was sampled in autumn 2010 (04/03/2010), and autumn 2011 (12/04/2011). Micalong Creek was not sampled in 2012 as a result of heavy rainfall and high flows. In 2011 only fyke nets could be used for sampling as a result of increased flows caused by heavy rainfall. A total of 13 Brown trout and four Rainbow trout were caught in 2010 ranging in size from 92 – 374 mm and 63 – 185 mm FL, respectively, (Figure 39). In 2011 a total of three Brown trout measuring 197, 210 and 275 mm and two Rainbow trout measuring 213 and 244 mm FL were caught at Micalong Ck.

Catches of Rainbow trout in fyke nets were comparable at each site between years with the exception of Downstream of Bendora, where catches in 2011 were two-fold that of 2010 and 2012 (Figure 41). Catches of Rainbow trout by electrofishing were comparable at each site between years with the exception of Downstream of Bendora, where catches in 2010 were four-fold that of 2010 and 2012 (Figure 42), and were mainly from 75 – 130 mm FL (which most likely represents 0+ to 1+ year old fish).

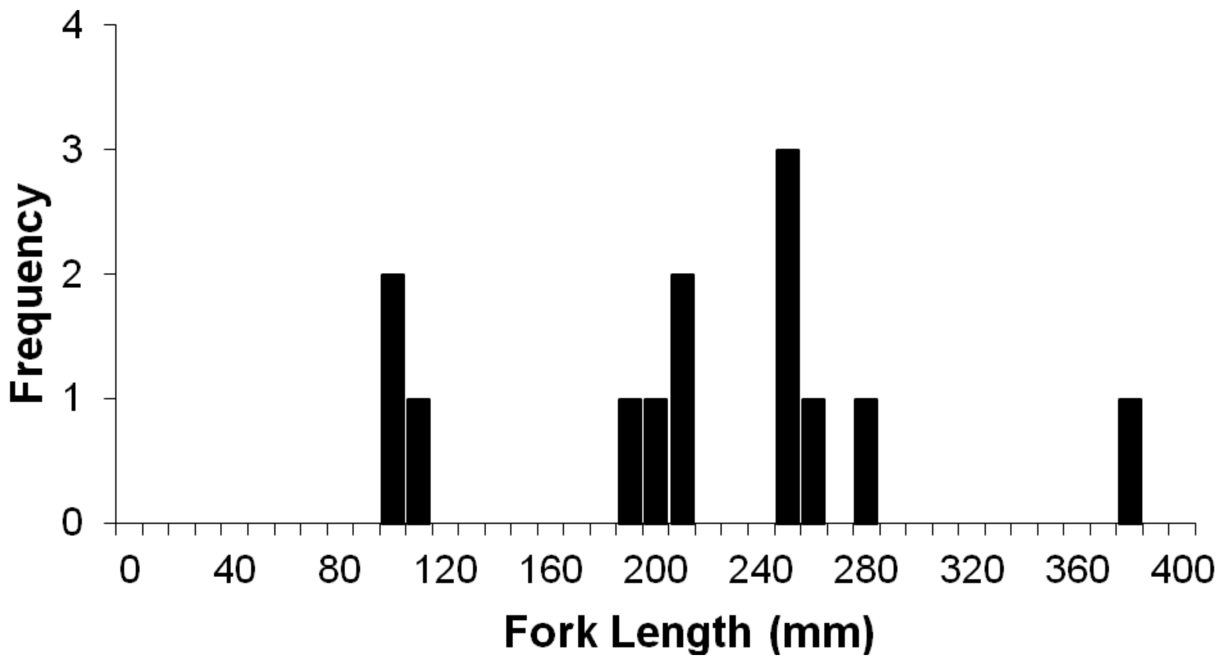


Figure 39. Length of Brown trout caught by two methods (fyke nets and electrofishing) from Micalong Creek in March 2010.

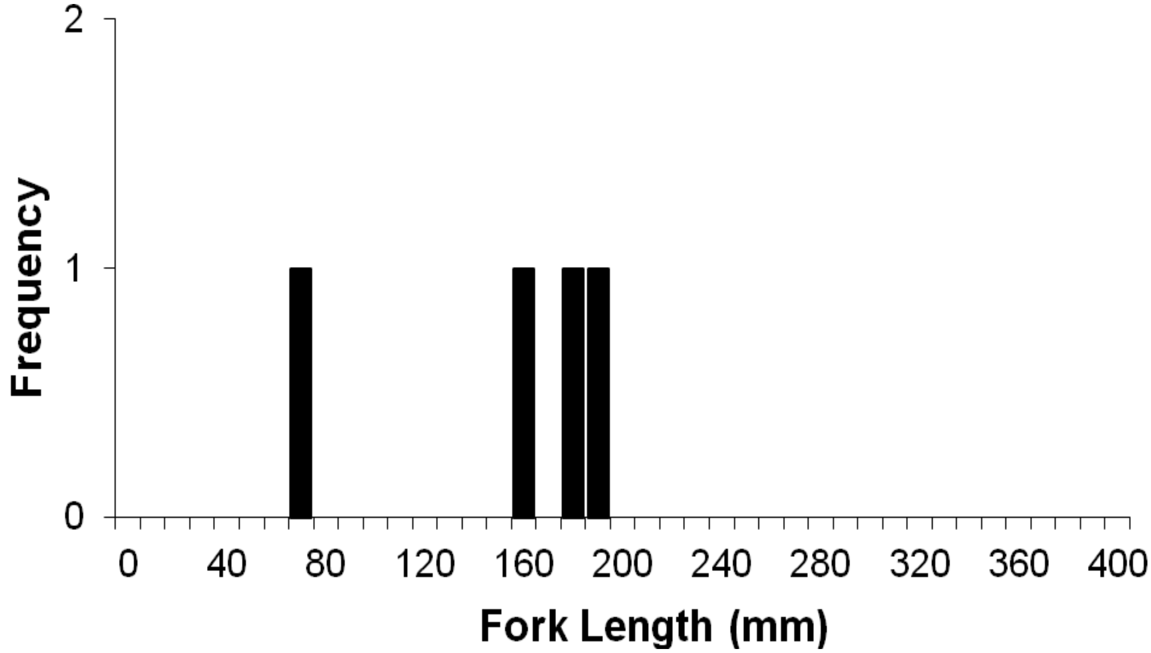


Figure 40. Length of Rainbow trout caught by two methods (fyke nets and electrofishing) from Micalong Creek in March 2010.

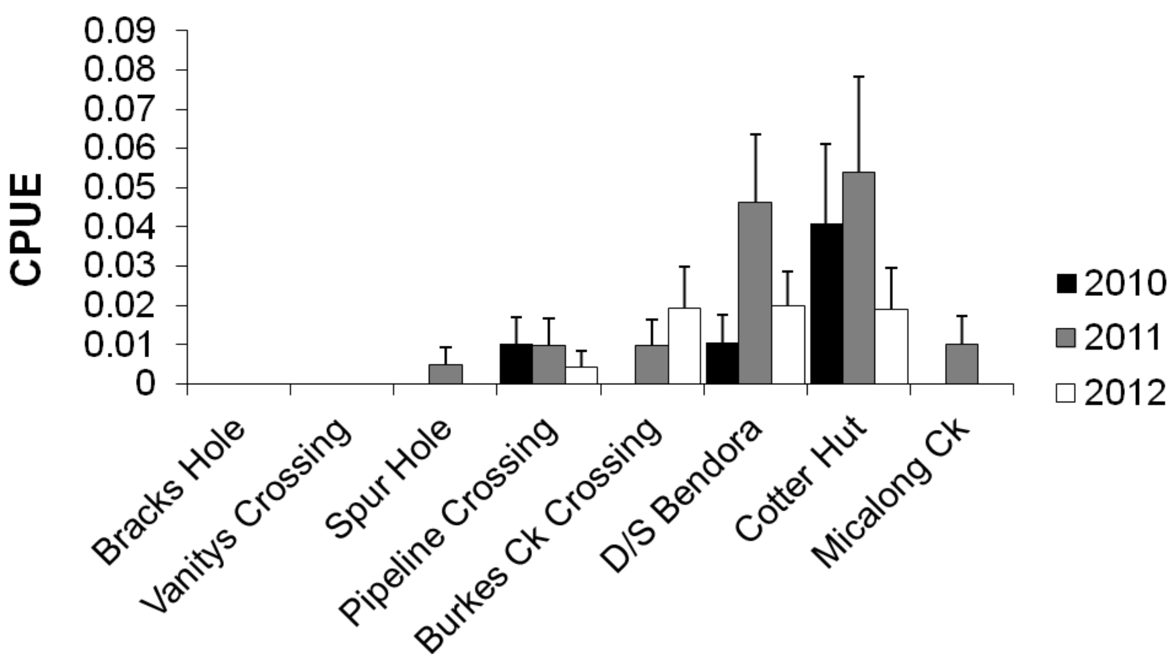


Figure 41. Mean (± SE) Rainbow trout captured per fyke-net hour (CPUE) in 2010, 2011 and 2012.

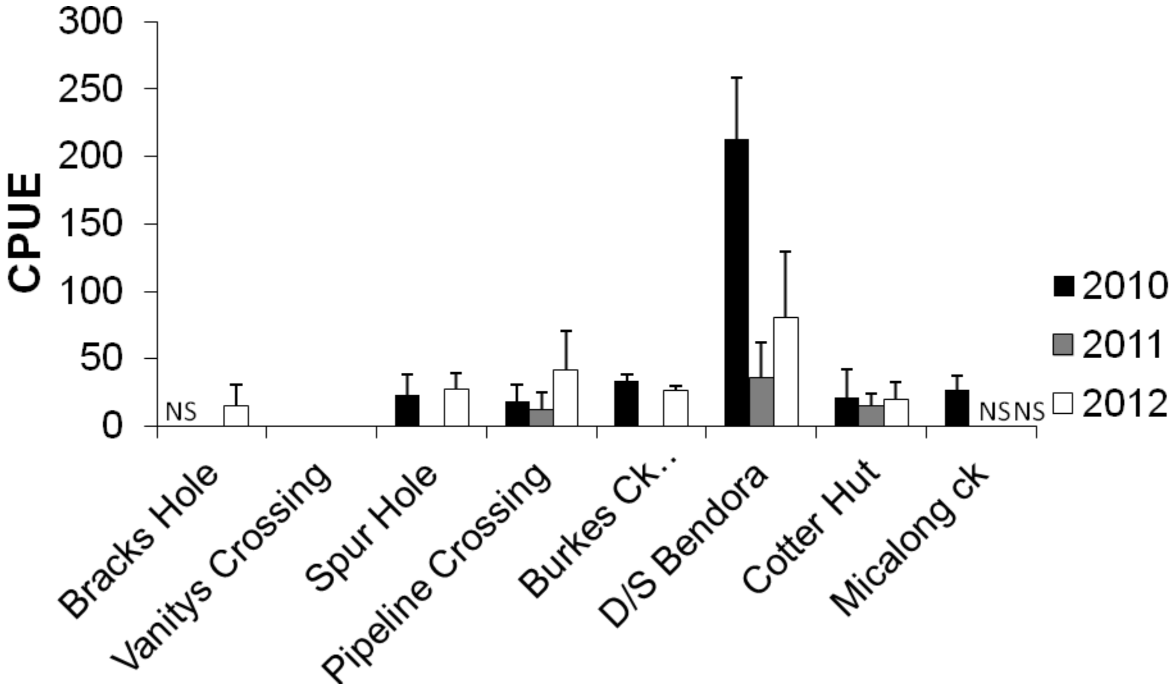


Figure 42. Mean (± SE) Rainbow trout captured per electrofishing hour (CPUE) in 2010, 2011 and 2012 (NS = not able to be sampled as a result of high flows).

Discussion

Rainbow trout abundance (as determined by fyke net captures and backpack electrofishing) was relatively stable throughout the study period in the Cotter River upstream of the Cotter Reservoir. The only exceptions for this were larger than normal captures of rainbow trout caught by electrofishing at the site immediately downstream of Bendora Dam in 2010. The large abundance was predominantly comprised of juvenile rainbow trout between 75 – 130 mm FL (0+ and 1+ year old).

The size composition of trout in the Cotter River upstream of Cotter Reservoir has been relatively stable of the three years of sampling with two main peaks in the length frequency histograms. The first peak (~80 – 140 mm FL) represents 0+ and 1+ year old Rainbow trout and the second peak (~200 – 300 mm FL) representing 2 – 4+ year old Rainbow trout (Lintermans and Rutzou 1990). The reference sites (Cotter Hut and Micalong Creek) both had relatively low catches of Rainbow trout which made it difficult to determine size composition trends. In general the size range was similar between the Cotter River (Bendora Dam to Cotter Reservoir) and the references sites with the majority of Rainbow trout captured being between 60 – 320 mm FL. The size of trout captured at the Cotter Hut reference site is similar to that recorded in the late 1980s by (Lintermans and Rutzou 1990) with few large fish (>300 mm FL) captured.

Conclusion

Rainbow trout abundance has been relatively stable over the three years, with some peaks in abundance occurring at some sites. Based on the results of the 3 years of baseline data there does not appear to be a significant number of large trout (>350 mm) present in the Cotter River upstream of Cotter Reservoir. The current sampling regime appears adequate in detecting a change in the abundance and size of trout in the Cotter River upstream of the ECD.

Recommendation

6.1 The current sampling design should remain unchanged for assessing abundance and size composition of trout in the Cotter River upstream of the ECD.

7) Will there be a significant increase in the levels of predation on Two-spined blackfish by trout in the Cotter River upstream of the ECD?

If trout abundance and size increases in the river upstream of the ECD (see Background to Question 6 above), it is possible that there will be increased predatory impacts on Two-spined blackfish. Monitoring of changes in the incidence of trout predation on Two-spined blackfish will guide potential management intervention to ameliorate such predation.

Sampling design

Trout (both rainbow and brown) were collected from eight riverine sites along the Cotter River. Sites consisted of the same six sites between Cotter Reservoir and Bendora Dam sampled for Question 6 (from most downstream to most upstream: Bracks Hole, Vanitys Crossing, Spur Hole, Pipeline Road. Crossing, Burkes Creek Crossing, Downstream of Bendora Dam) and two reference sites upstream of Corin Reservoir (Gallipoli Flat and Cotter Hut). Sites were sampled by backpack electrofishing. Sampling effort at each site consisted of backpack electrofishing 1km of river, or the collection of 20 trout (>150 mm – size at which trout gape would be theoretically sufficient to ingest a juvenile Two-spined blackfish), whichever occurs first. Sampling was conducted seasonally. Trout stomachs were removed in the field, and contents stored in 100% ethanol to be later sorted under stereo-microscope in the laboratory. Fish and crayfish found in stomach contents were identified to species (where possible), with insects classified as either aquatic or terrestrial based on their origin. Estimates of the composition of dietary items were made based on percent volume of each item of the total volume of all items. Stomach contents were retained to allow future genetic identification of unidentified fish remains. Sampling was not able to be undertaken in spring 2010 and 2011 as a result of high flows in Cotter River caused by heavy rainfall.

Results

For the three seasons combined in 2010, 198 trout comprising both species (190 Rainbow trout and eight Brown trout), were kept for stomach content analysis (

Table **12**). Autumn had the highest number of trout stomachs examined (81) followed by summer (80) and winter (37) with fish sizes ranging from 90 – 502 mm FL for Rainbow trout and 153 – 513 mm FL for Brown trout (see Figure 43 for length frequency of Rainbow trout examined for each season). For Rainbow trout, aquatic insects were the most common dietary item found, present in 94% of stomachs for all seasons combined. Following aquatic invertebrates, terrestrial invertebrates were the next most common (79%), followed by plant material (7%) and fish (1%). A small number of trout stomachs were found to be empty (2%). One rainbow trout captured in Autumn at Pipeline Crossing had a 26cm juvenile Tiger snake in its stomach (Clear 2011). Of the visually identifiable fish remains that were examined, two Two-spined blackfish and one Rainbow trout were identified. No non-identifiable fish remains were encountered. The length of the Two-spined blackfish recorded in trout stomach that was able to be measured was 156 mm.

For the three seasons combined in 2011, 290 trout comprising both species (288 Rainbow trout and two Brown trout), were kept for stomach content analysis (Table 13). Autumn had the highest number of stomachs examined (110) followed by winter (107) and summer (73) with sizes ranging from 148 – 460 mm FL for Rainbow trout and 167 – 285 mm FL for Brown trout (see Figure 44 for length frequency of Rainbow trout kept for each season). For Rainbow trout aquatic invertebrates were the most common dietary item found, present in 99% of stomachs for all seasons combined. Following aquatic invertebrates, terrestrial invertebrates were the next most common (78%), followed by plant material (21%) and fish (1%). A small number of fish stomachs were found to be empty (1%). Three Two-spined blackfish were found, with lengths of 36, 166 and 141 mm TL.

In 2012 all four seasons were sampled with 222 trout comprising both species (216 Rainbow trout and six Brown trout) kept for stomach content analysis (Table 14). Summer had the highest number of stomachs examined (96) followed by winter (46), autumn (43) and spring (37) with sizes ranging from 150 – 388 mm FL for Rainbow trout and 160 – 500 mm FL for Brown trout (see Figure 45 for length frequency of Rainbow trout kept for each season). For Rainbow trout aquatic invertebrates were the most common dietary item found, present in 100% of stomachs for all seasons combined. Following aquatic invertebrates, terrestrial invertebrates were the next most common (76%) followed by plant material (27%). Only one fish from all seasons combined was found with an empty stomach. Lizard remains were found in two separate stomachs and unidentified fish remains were found in one stomach. The single Brown trout caught in spring had two Rainbow trout in its stomach, measuring 140 and 180 mm FL.

Table 12. Summary of number of trout stomachs analysed for each site for each of the three seasons sampled in 2010 (note: Spring sample could not be obtained as a result of high river flows).

Summer		
Site	Rainbow trout	Brown trout
Bracks Hole	0	0
Burkes Ck. Xing	18	2
Cotter Hut	22	0
D/S Bendora	16	2
Gallipoli Flat	16	0
Pipeline Rd. Xing	2	0
Spur Hole	2	0
Vanitys Xing	0	0
Total	**76**	**4**
Size range (mm)	**90–330**	**227–513**

Autumn		
Site	Rainbow trout	Brown trout
Bracks Hole	0	0
Burkes Ck. Xing	1	0
Cotter Hut	20	0
D/S Bendora	21	1
Gallipoli Flat	21	0
Pipeline Rd Xing	1	0
Spur Hole	3	0
Vanitys Xing	4	0
Total	**80**	**1**
Size range (mm)	**152–365**	**206**

Winter		
Site	Rainbow trout	Brown trout
Bracks Hole	0	0
Burkes Ck. Xing	3	2
Cotter Hut	2	0
D/S Bendora	19	1
Gallipoli Flat	5	0
Pipeline Rd. Xing	2	0
Spur Hole	3	0
Vanitys Xing	0	0
Total	**34**	**3**
Size range (mm)	**150–502**	**153–471**

Table 13. Summary of number of trout stomachs analysed for each site for each of the three seasons sampled in 2011.

Summer		
Site	Rainbow trout	Brown trout
Bracks Hole	0	0
Burkes Ck. Xing	5	0
Cotter Hut	16	0
D/S Bendora	20	0
Gallipoli Flat	20	0
Pipeline Rd. Xing	7	0
Spur Hole	0	0
Vanitys Xing	5	0
Total	**73**	
Size range (mm)	**148–460**	

Autumn		
Site	Rainbow trout	Brown trout
Bracks Hole	10	0
Burkes Ck. Xing	20	0
Cotter Hut	20	0
D/S Bendora	15	0
Gallipoli Flat	20	0
Pipeline Rd Xing	8	0
Spur Hole	9	0
Vanitys Xing	8	0
Total	**110**	
Size range (mm)	**150–360**	

Winter		
Site	Rainbow trout	Brown trout
Bracks Hole	16	0
Burkes Ck. Xing	20	0
Cotter Hut	9	0
D/S Bendora	18	1
Gallipoli Flat	14	0
Pipeline Rd. Xing	9	0
Spur Hole	9	0
Vanitys Xing	10	1
Total	**105**	**2**
Size range (mm)	**150–413**	**167–285**

Table 14. Summary of number of trout stomachs analysed for each site for each of the three seasons sampled in 2012.

Summer		
Site	Rainbow trout	Brown trout
Bracks Hole	0	0
Burkes Ck. Xing	18	2
Cotter Hut	13	0
D/S Bendora	15	0
Gallipoli Flat	20	0
Pipeline Rd. Xing	16	0
Spur Hole	10	0
Vanitys Xing	1	1
Total	**93**	**3**
Size range (mm)	**150–360**	**245–475**

Autumn		
Site	Rainbow trout	Brown trout
Bracks Hole	1	0
Burkes Ck. Xing	6	0
Cotter Hut	8	0
D/S Bendora	16	0
Gallipoli Flat	5	0
Pipeline Rd Xing	4	0
Spur Hole	3	0
Vanitys Xing	0	0
Total	**43**	**0**
Size range (mm)	**156–388**	

Winter		
Site	Rainbow trout	Brown trout
Bracks Hole	4	0
Burkes Ck. Xing	7	0
Cotter Hut	0	0
D/S Bendora	18	2
Gallipoli Flat	0	0
Pipeline Rd Xing	3	0
Spur Hole	4	0
Vanitys Xing	8	0
Total	**44**	**2**
Size range (mm)	**150–369**	**160–234**

Spring		
Site	Rainbow trout	Brown trout
Bracks Hole	0	0
Burkes Ck. Xing	8	0
Cotter Hut	0	0
D/S Bendora	8	1
Gallipoli Flat	3	0
Pipeline Rd Xing	7	0
Spur Hole	6	0
Vanitys Xing	4	0
Total	**36**	**1**
Size range (mm)	**150–332**	**500**

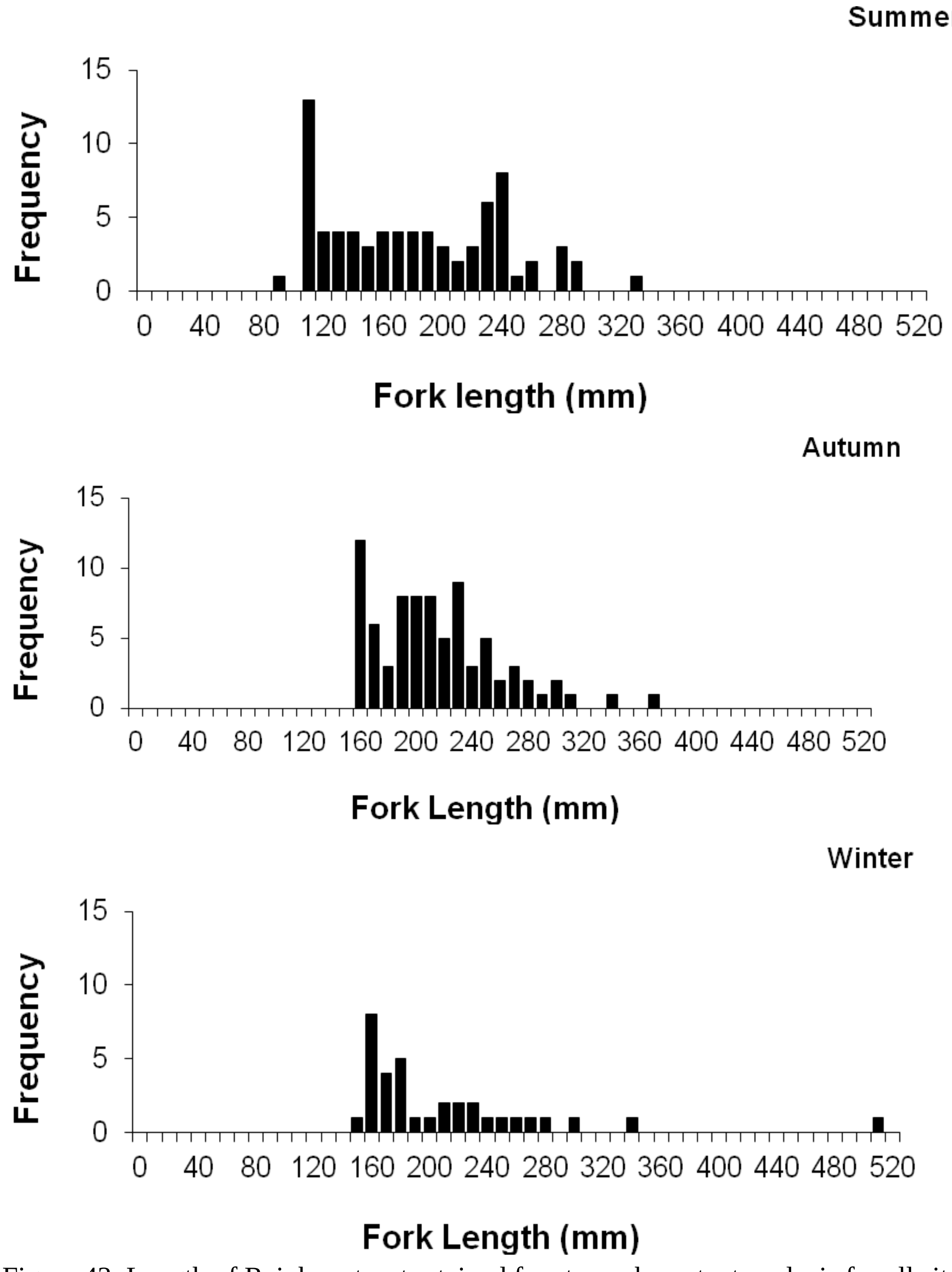


Figure 43. Length of Rainbow trout retained for stomach content analysis for all sites combined per season 2010.

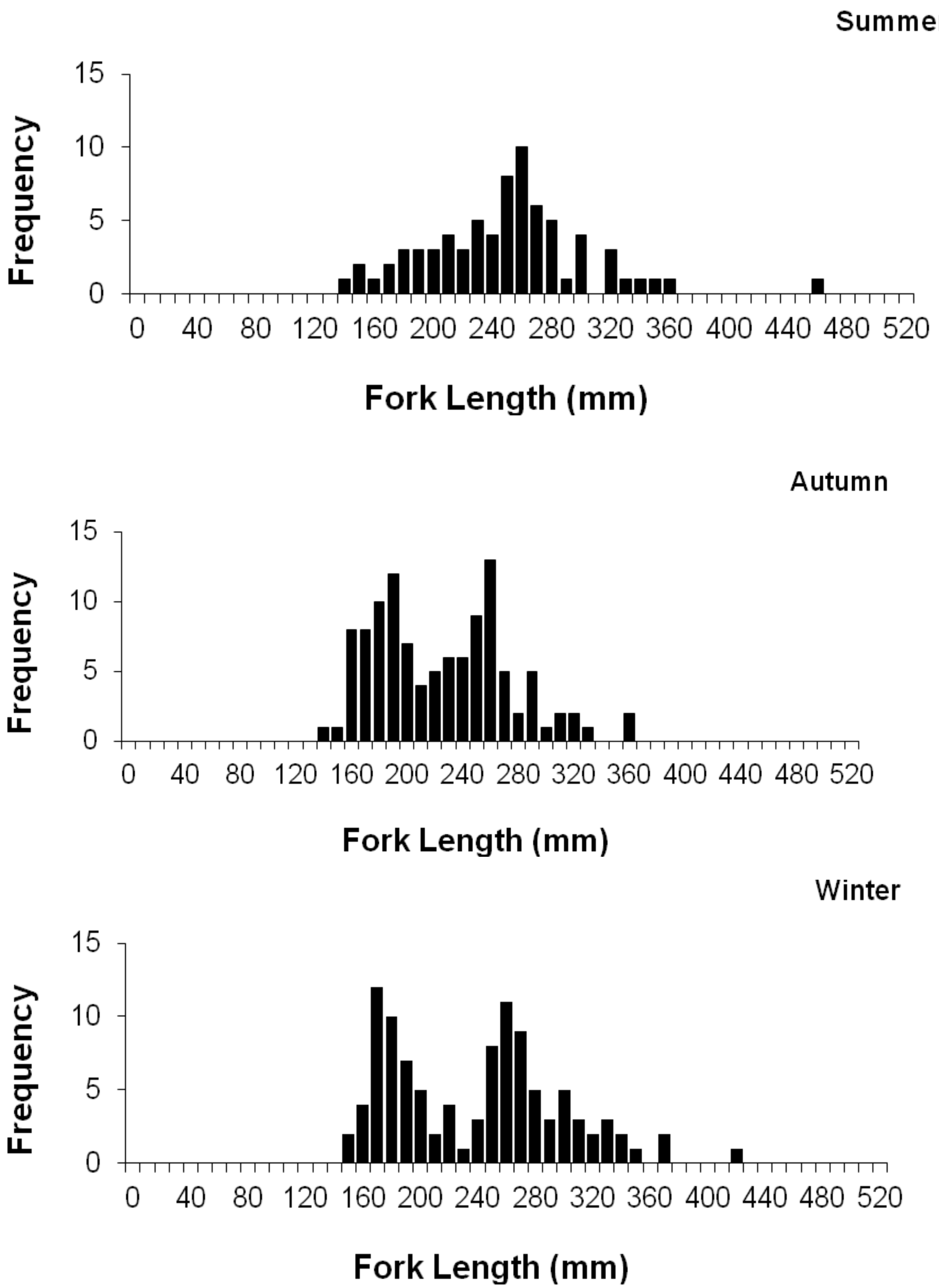
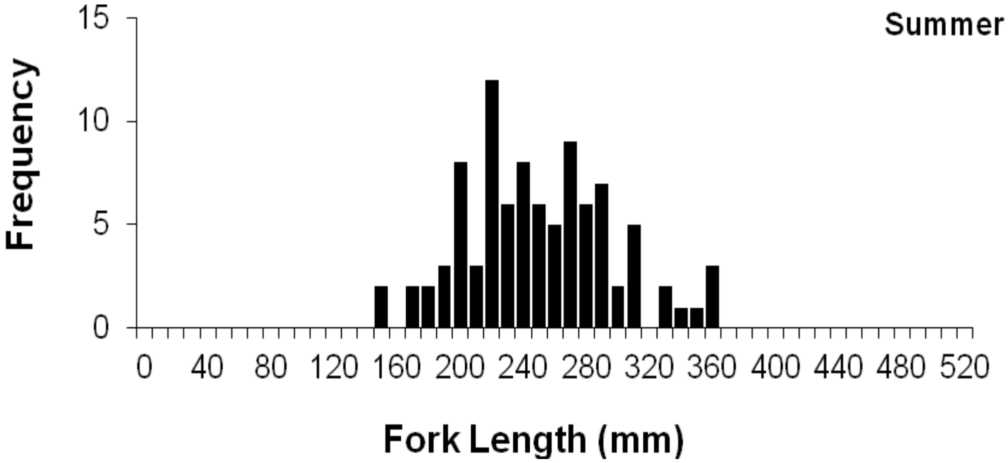
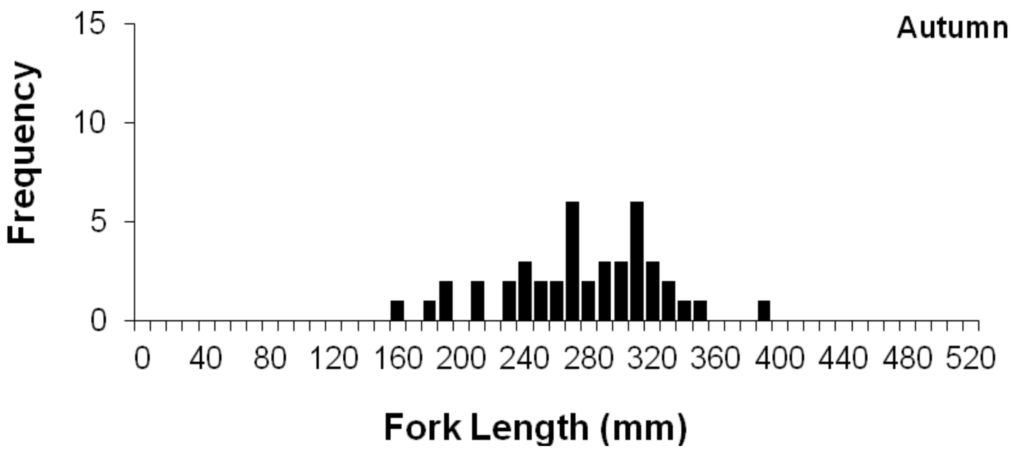
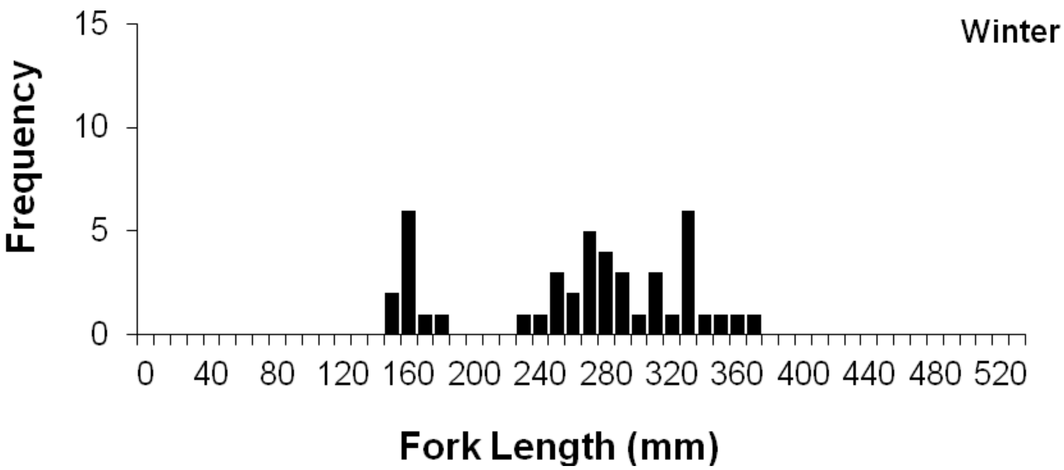


Figure 44. Length of Rainbow trout retained for stomach content analysis for all sites combined per season 2011.


Summer
Frequency
15
10
5
0
0 40 80 120 160 200 240 280 320 360 400 440 480 520
Fork Length (mm)


Autumn
Frequency
15
10
5
0
0 40 80 120 160 200 240 280 320 360 400 440 480 520
Fork Length (mm)


Winter
Frequency
15
10
5
0
0 40 80 120 160 200 240 280 320 360 400 440 480 520
Fork Length (mm)

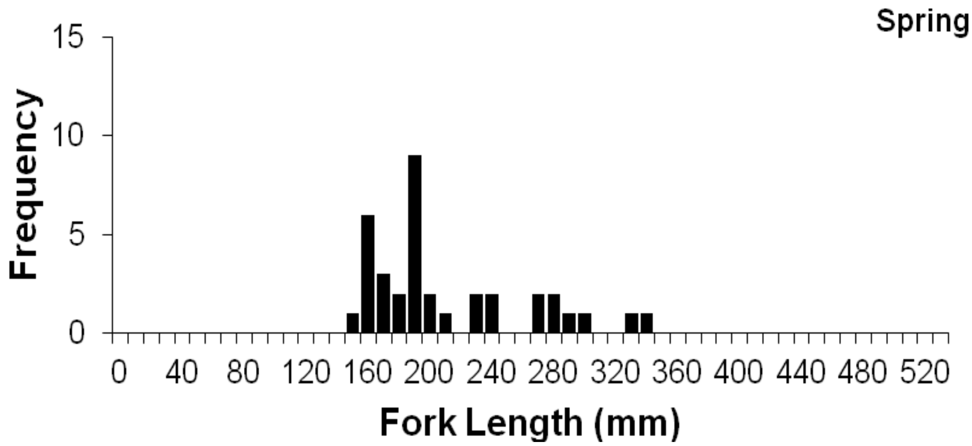


Figure 45. Length of Rainbow trout retained for stomach content analysis for all sites combined per season 2012.

Discussion

The sampling design of trying to minimise fieldwork costs by combining the trout sampling for Q4 (Macquarie perch predation by trout) and Q7 was counterproductive. Delaying the spring sample for Q7 to as late as possible in spring (to overlap with Macquarie perch larval presence) meant that when heavy rains (and associated high river flows) occurred in spring and early summer in 2010 and 2011, we were unable to reschedule a spring sample.

Overall the predation rate of Rainbow trout on Two-spined blackfish was low in two of the three years, and non-detectable in the third. Fish were rarely found in the stomachs of the trout examined, being present in less than 1% of stomachs in all three years. A total of only five instance of predation of Two-spined blackfish were observed, of which two instances were from reference sites. All trout found to contain fish in the stomachs were greater than 250 mm FL, the length at which Ebner *et al.* (2007) found trout to start piscivory in the Cotter Reservoir. However, previous research in the Cotter Catchment record Rainbow trout as small as 131 mm FL preying on Two-spined blackfish (Lintermans unpubl. data). The majority of Two-spined blackfish recorded from trout stomachs were adult fish (>120 mm TL, (Lintermans 1998), reinforcing that all size classes of Two-spined blackfish (YOY, juveniles, adults) are potentially susceptible to trout predation.

Conclusion

The results of the three years of stomach contents analysis of trout in Cotter River upstream of Cotter Reservoir indicates that predation rates of Two-spined blackfish are relatively stable between years. The current sampling regime appears to be adequate to detect changes in the predation rate into the future.

Recommendation

7.1 The current sampling design should remain unchanged for assessing levels of predation on Two-spined blackfish by trout in the Cotter River upstream of the ECD.

8) Will there be significant changes in the abundance and distribution of the Macquarie perch population in the Cotter River above and below Vanitys Crossing?

The construction of Vanity's Crossing fishway in 2001 has allowed the Macquarie perch population to expand upstream of this road crossing. Some recruitment has been detected upstream of Vanity's Crossing, but it is unknown whether the population is self sustaining. Planned remediation of fish passage barriers at Pipeline Road Crossing and Burkes Creek Crossing (ACTEW Corporation 2009b) will open up further spawning habitat for the species. The remediation of these two road crossings is an offset under the ECD PER to compensate for the inundation of existing Macquarie perch spawning habitat by the ECD. Success of remediation of the two upstream road crossings is largely reliant on the continued success of the Vanity's Crossing fishway. Monitoring is required to determine the success of fish passage remediation at the two upstream road crossings and the affects of improved access to additional spawning habitat by the riverine Macquarie perch population.

Sampling design

Six riverine sites were sampled between Cotter Reservoir and Bendora Reservoir (the same six sites as sampled for Q6 and Q7), these were (in order from most downstream to most upstream); Bracks Hole, Vanitys Crossing, Spur Hole, Pipeline Road. Crossing, Burkes Creek. Crossing and Downstream of Bendora Dam. A reference site, Kissops Flat on the upper Murrumbidgee River was also sampled. Sites were sampled annually with fyke nets and backpack electrofishing in summer to autumn (8/2/2010 – 22/4/2010, 07/03/2011 – 05/05/2011 and 18/01/2012 – 16/04/2012). At each site, three fyke nets were set in each of four pools and four 30 m section of river were sampled by backpack electrofishing. Vanitys Crossing on the Cotter River, and Kissops Flat on the Murrumbidgee River have only one large pool, so all 12 fyke nets were set in the one pool at each of these sites. Each site was sampled for a single night.

Results

In 2010, a total of 164 Macquarie perch were caught from all six sites on the Cotter River combined using both methods of capture. They ranged in size from 26 – 308 mm TL (Figure 46). In 2011, a total of 230 Macquarie perch were caught from all six sites on the Cotter River combined ranging in size from 42 – 275 mm TL (Figure 47). In 2012, a total of 75 Macquarie perch were caught from all six sites on the Cotter River combined using both methods of capture. They ranged in size from 43 – 238 mm TL (Figure 48).

At Kissops Flat, a total of 15 Macquarie perch were caught in 2010 ranging in size from 46 –281 mm TL (Figure 49). In autumn 2011 a total of 96 Macquarie perch were caught ranging in size from 43 – 390 mm TL (Figure 50), and in 2012 a total of 61 Macquarie perch were caught ranging in size from 44 – 417 mm TL (Figure 51).

Strong abundance of YOY in the 2010 Cotter catchment sample (fish < 80 mm TL) flowed through to the 2011 sample (fish between 90-150 mm TL) (Figure 46 & Figure 47). Abundance of YOY in the 2011 Cotter catchment sample was much lower than in the 2010 sample. In 2010, catch per unit effort by fyke nets was relatively similar for the three riverine sites in the Cotter River where Macquarie perch are known to be present in good numbers (i.e. Bracks Hole, Vanitys Crossing and Spur Hole), though they were detected in low numbers at both Pipeline Rd. Crossing and Burkes Ck Crossing (Table 15). At the external reference site (Kissops Flat) numbers of Macquarie perch caught were modest compared to the three sites on the Cotter River in 2010 (Table 15). In 2011, similar CPUE for Macquarie perch was observed as for 2010, with the exception of Vanitys Crossing on the Cotter River, and Kissops Flat on the upper Murrumbidgee River, which had two-fold and six-fold increases, respectively, in 2011 (Table 15). The big increase in catch of Macquarie perch at the Kissops Flat site in 2011 can be largely attributed to strong YOY recruitment (reflected in large numbers of individuals < 100 mm (Table 15 & Figure 50). Catch rates of Macquarie perch were similar between 2010 and 2011 across most sampling sites, with the exception of Vanitys Crossing on the Cotter River and Kissops Flat on the upper Murrumbidgee River, which both had higher catch rates in 2011 (Figure 52). In 2012, the abundance of YOY Macquarie perch (fish < 80 mm TL) in the Cotter River was slightly higher than 2011 but the overall catch was a third of the previous year and half of the 2010 catch rate (Figure 48). The CPUE was down at all sites in 2012 compared to 2011 except for Pipeline Rd. Crossing which had almost three times the catch rate than the previous year (Figure 52 & Table 15). It was a good year for Macquarie perch in 2012 at Kissops Flat with the highlight being the dominance of the catch by YOY fish (<80 mm TL), however the previous year's abundance of YOY did not flow through to 2012 as only a few fish in the 90 – 150 mm size class were captured (Figure 51). The CPUE was down by a third at Kissops Flat in 2012 compared to the previous year but was still fourfold higher compared to 2010 (Table 15 & Figure 52). All fish were captured by fyke nets only in 2011 and 2012 with 2010 being the only year where fish were caught by both fyke nets and electrofishing (Figure 53).

Table 15. Mean CPUE (± SE) and mean length (mm TL ± SE) of Macquarie perch caught by fyke nets in the sites on the Cotter River and a site on the Murrumbidgee River (Kissops Flat) in 2010 and 2011. CPUE is no. fish per fyke-net hour

	2010		2011		2012	
	CPUE	Length	CPUE	Length	CPUE	Length
Bracks Hole	0.27 ± 0.06	91 ± 7	0.27 ± 0.08	131 ± 5	0.12 ± 0.02	86 ± 12
Vanitys Xing	0.22 ± 0.08	145 ± 14	0.55 ± 0.24	151 ± 5	0.22 ± 0.06	127 ± 6
Spur Hole	0.31 ± 0.06	94 ± 7	0.23 ± 0.09	124 ± 5	0.02 ± 0.01	188 ± 10
Pipeline Rd. Xing	0.01 ± 0.01	197 ± 45	0.03 ± 0.02	139 ± 13	0.08 ± 0.08	175
Burke Ck. Xing	0.005 ± 0.01	135	0.004 ± 0.004	275	0	0
D/S of Bendora	0	0	0	0	0	0
Kissops Flat	0.07 ± 0.02	122 ± 20	0.44 ± 0.14	81 ± 6	0.30 ± 0.14	74 ± 8

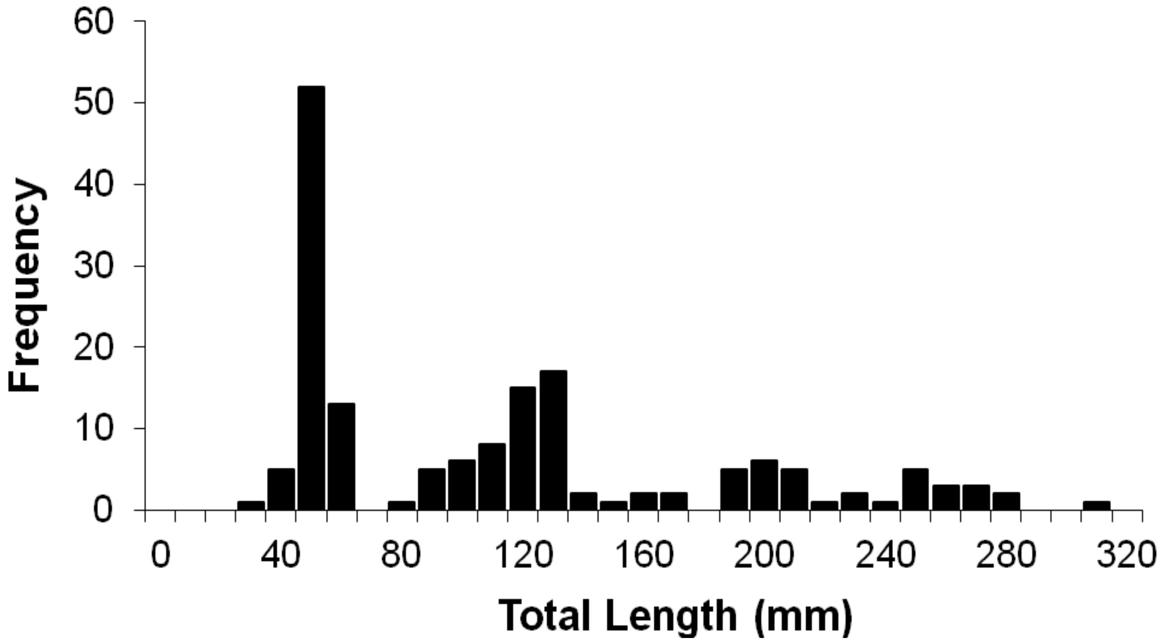


Figure 46. Length of Macquarie perch caught from six sites between Cotter Reservoir and Bendora Dam using fyke nets and backpack electrofishing in February 2010.

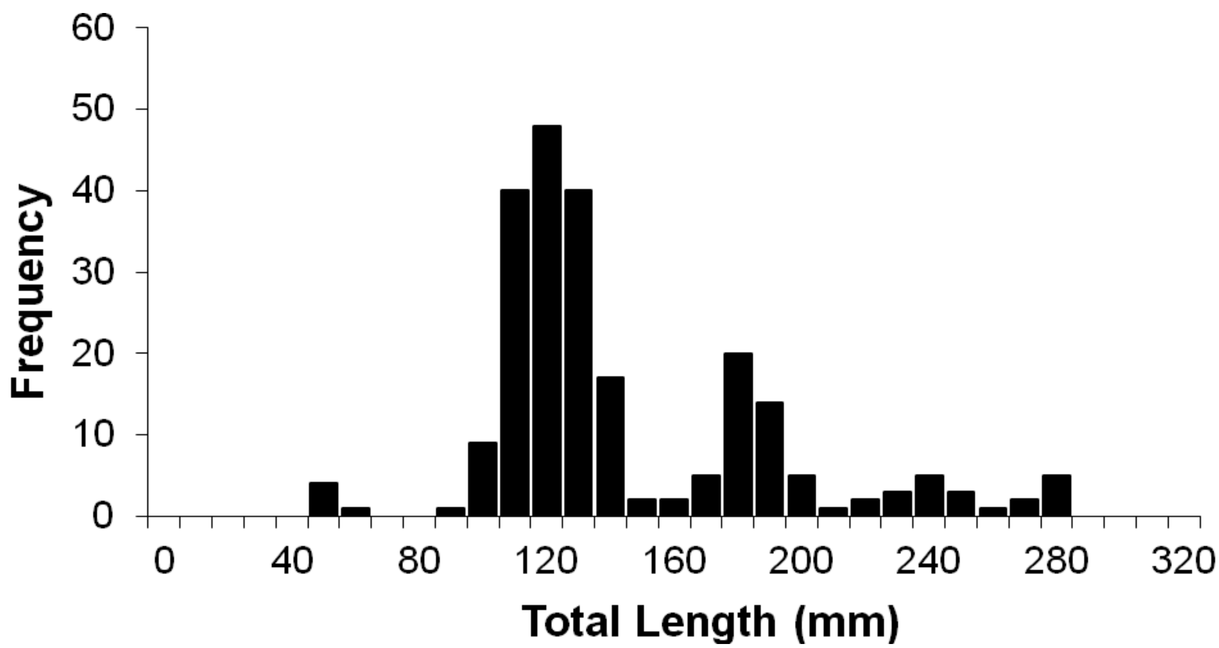


Figure 47. Length of Macquarie perch caught from six sites between Cotter Reservoir and Bendora Dam using fyke nets in March 2011. No Macquarie perch were captured by backpack electrofishing.

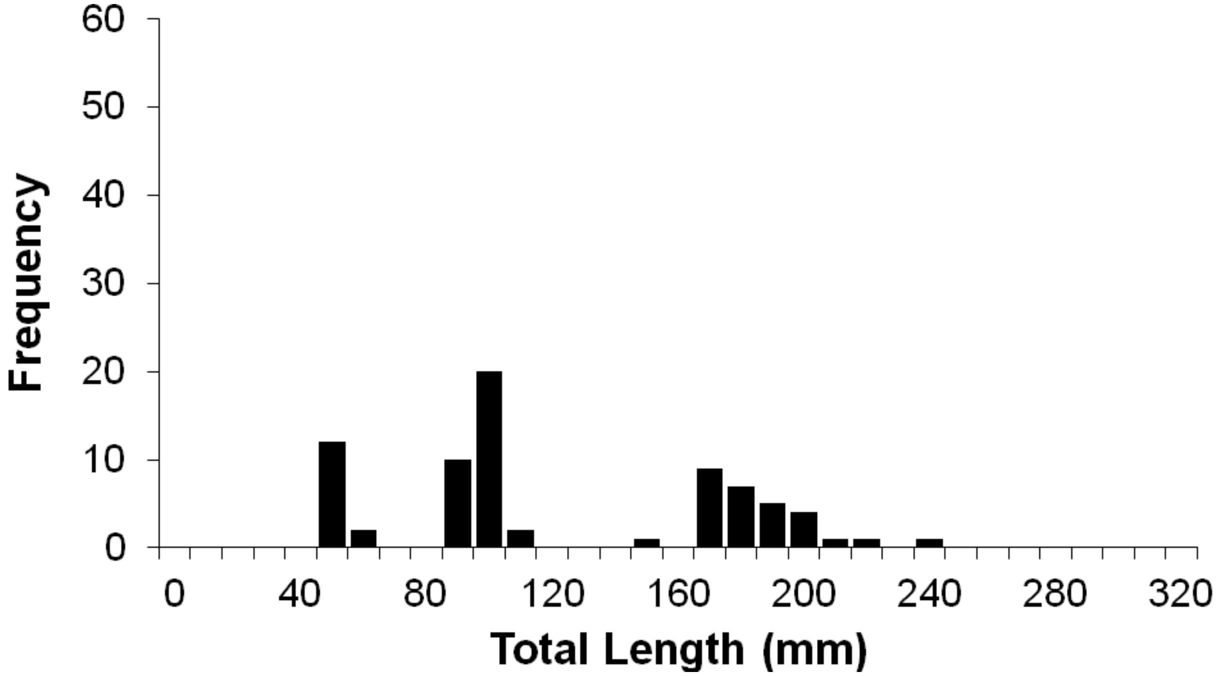


Figure 48. Length of Macquarie perch caught from six sites between Cotter Reservoir and Bendora Dam using fyke nets in February 2012. No Macquarie perch were captured by backpack electrofishing.

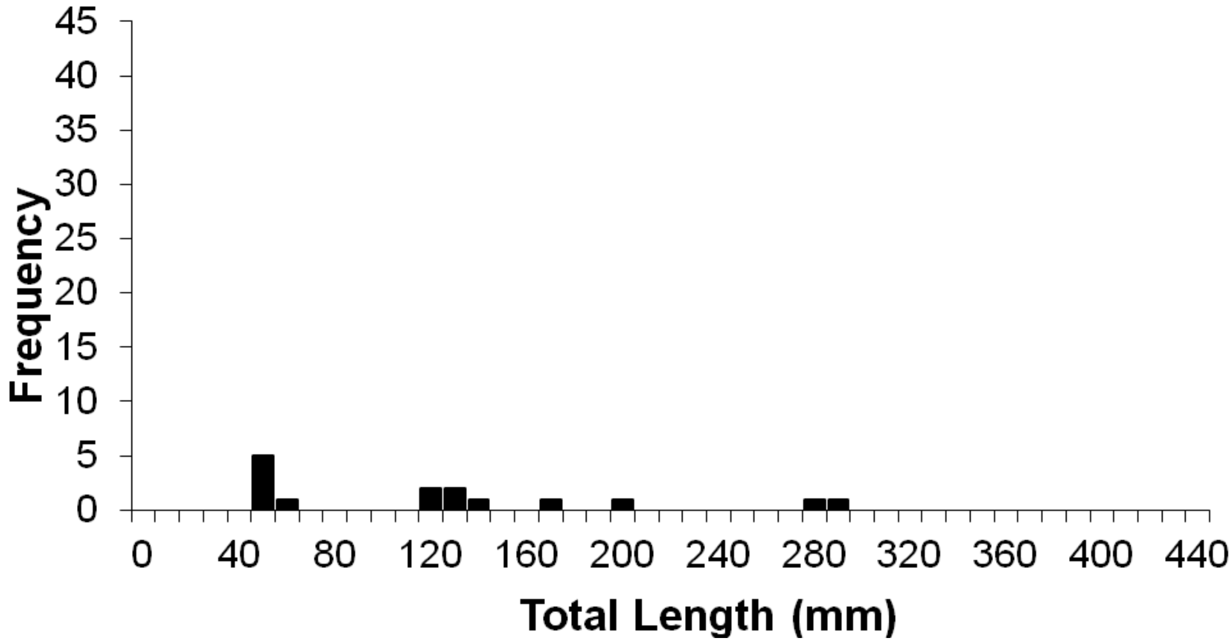


Figure 49. Length of Macquarie perch caught from Kissops Flat using fyke nets and backpack electrofishing in April 2010.

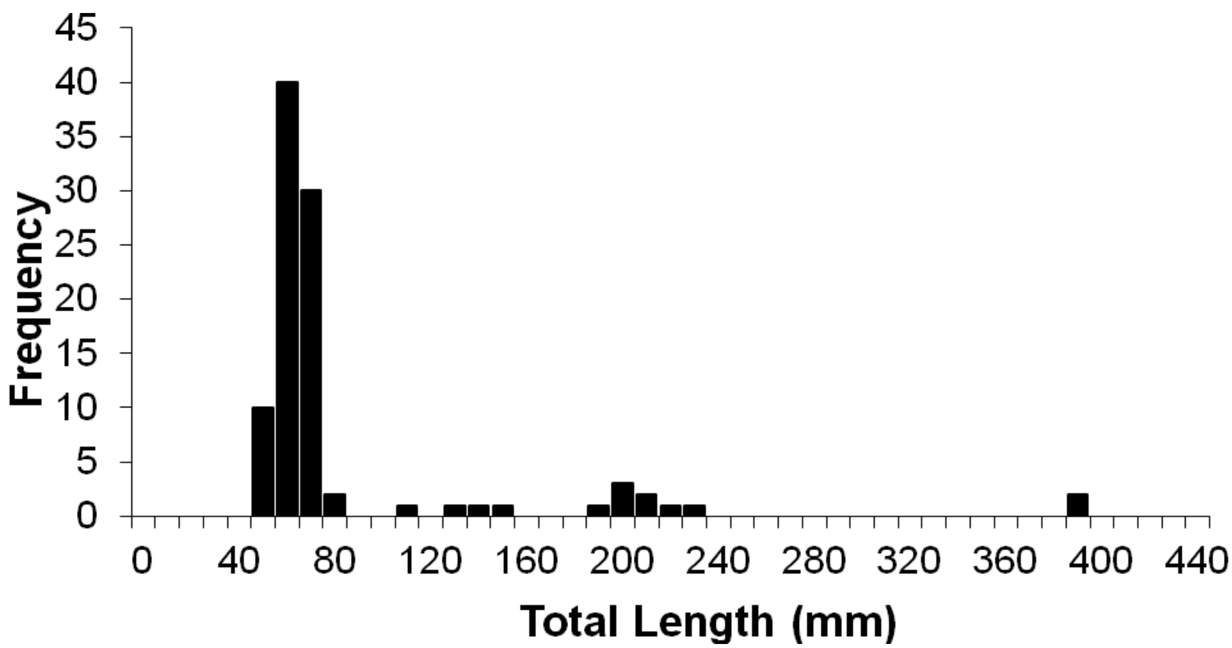


Figure 50. Length of Macquarie perch caught from Kissops Flat using fyke nets in May 2011. No Macquarie perch were captured by backpack electrofishing.

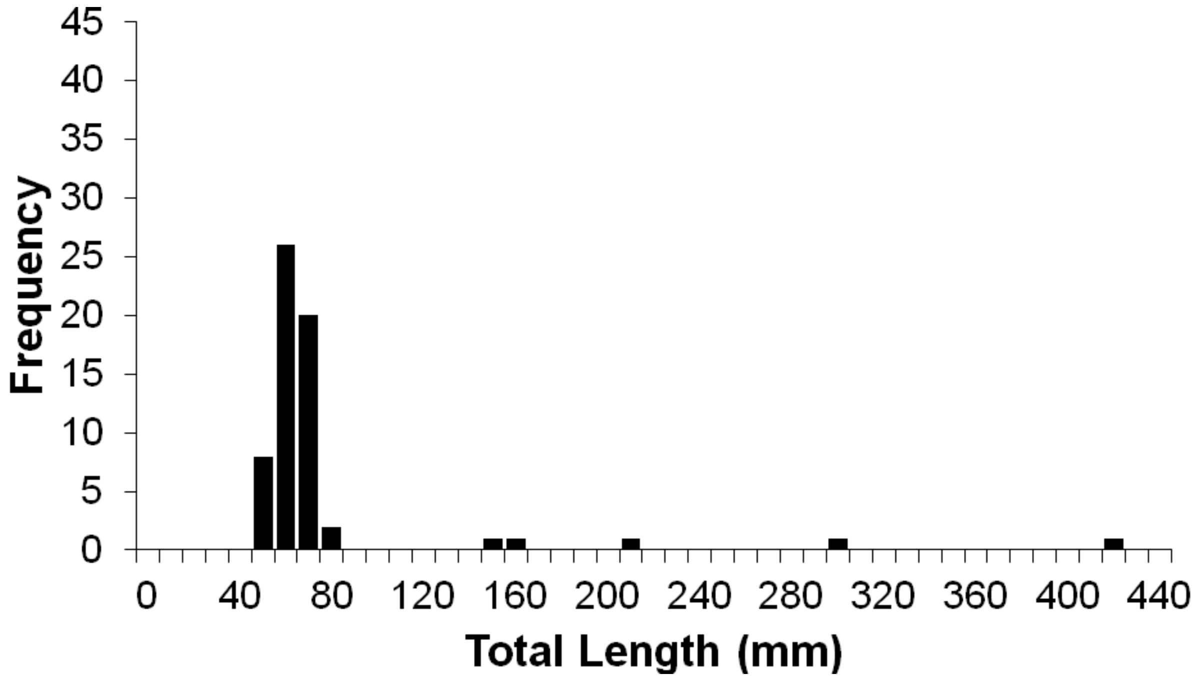


Figure 51. Length of Macquarie perch caught from Kissops Flat using fyke nets in April 2012. No Macquarie perch were captured by backpack electrofishing.

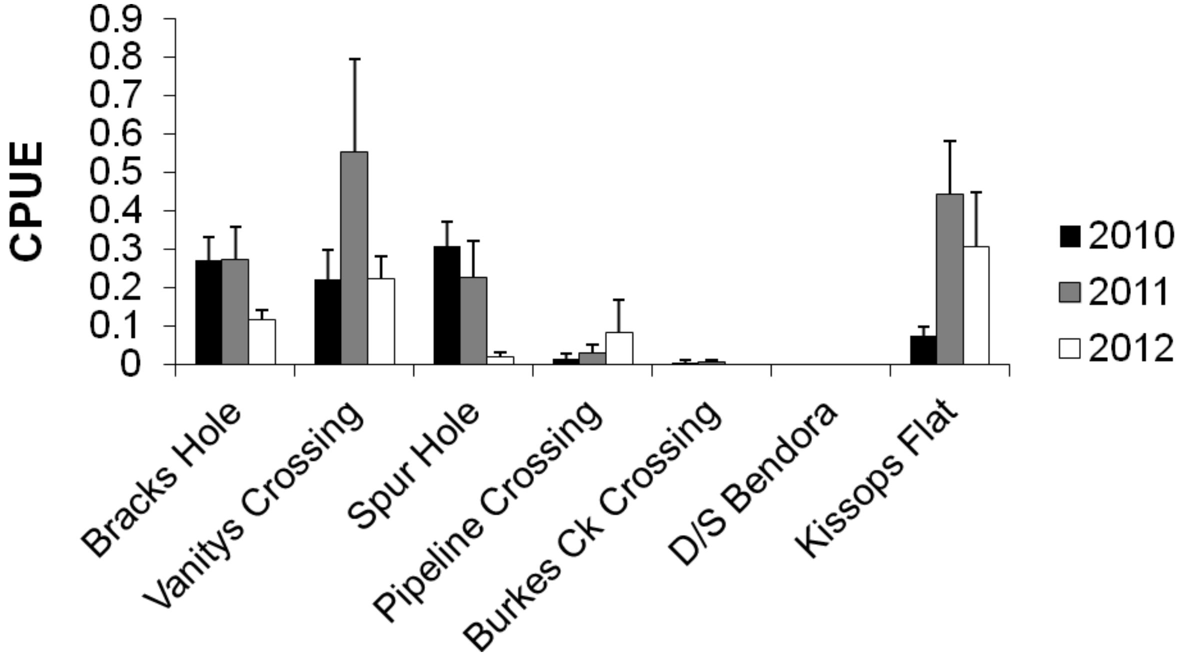


Figure 52. Mean (± SE) Macquarie perch captured per fyke-net hour (CPUE).

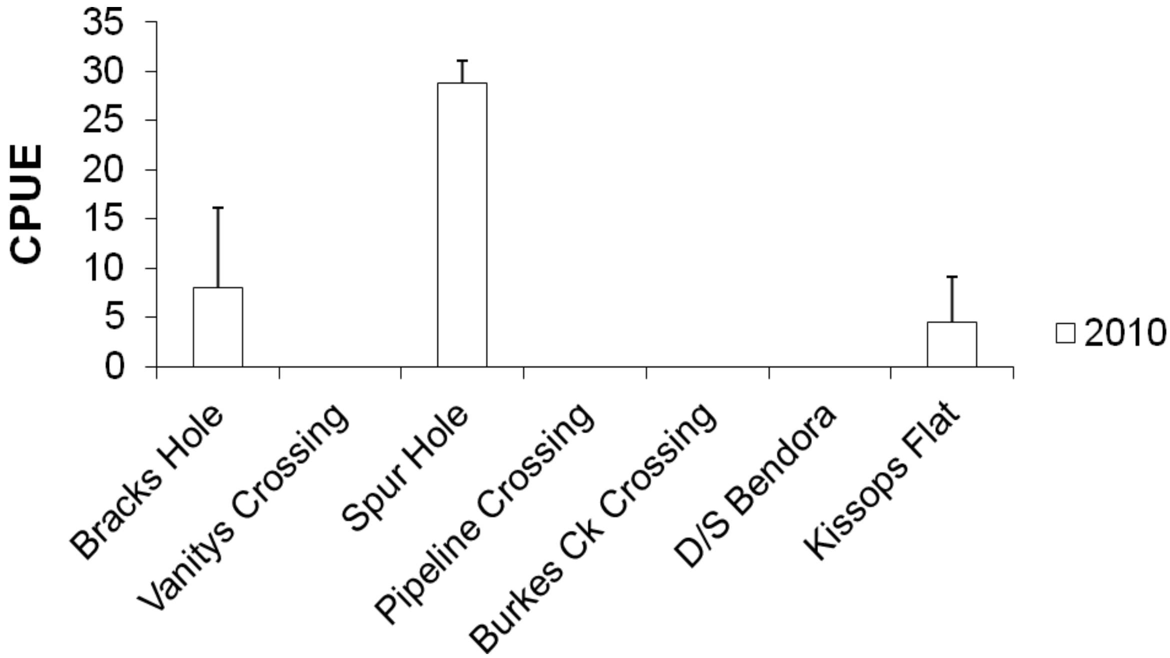


Figure 53. Mean (± SE) Macquarie perch captured per electrofishing hour (CPUE). (Note: no Macquarie perch were captured by electrofishing in 2011 and 2012).

Discussion

Abundance of Macquarie perch as detected by fyke netting fluctuated between years at both the Cotter River sites and the reference site (Kissops Flat). Abundances of Macquarie perch were similar in 2010 and 2011 for the Cotter River sites with the exception of Vanitys Crossing where higher abundances were observed in 2011. The increased catch of Macquarie perch in 2011 at Vanitys Crossing comprised mainly 1+ and 2+ individuals (98 of 116) indicating that individuals from 2009 and 2010 spawning seasons was important. Two sites (one below and one above Vanitys Crossing) recorded low abundances of Macquarie perch in 2012, with Spur Hole (above Vanitys Crossing) particularly low compared to previous years. Abundance of Macquarie perch in 2010 at the reference site was low (approximately one quarter of that found in 2011 and 2012) with few YOY recorded. However abundance increased in 2011 and 2012 due largely to the increased prevalence of young-of-year individuals indicating successful spawning had been undertaken the previous spring/summer. The large variation in abundance of Macquarie perch found at the reference site may result in the current study design having poor power to detect a true change at the impact site (i.e. Cotter River). The addition of a second reference site could be added to the sampling regime to act as a buffer to localised changes in the abundance of Macquarie perch at the current reference site. This would reduce the chances of a detection of a false change in the abundance of Macquarie perch at the treatment site and may prevent unnecessary management action or may prevent lack of management action should there be a true decline in abundance undetected at the treatment site.

Macquarie perch were detected at all sites in the Cotter River as far upstream as Burkes Creek Crossing in 2010 and 2011, but were not present at this site in 2012. It is likely that Macquarie perch were present at this site during 2012, but at such low densities that they were not detected by the standard sampling regime applied. The catch per unit effort declined with distance upstream of Vanitys Crossing, suggesting that whilst a population is now well established immediately upstream of Vanitys Crossing, it is still colonising the reaches upstream. The recent construction of the Pipeline Road Crossing rock-ramp fishway will facilitate the upstream expansion of the Macquarie perch population. A study is currently being undertaken by the University of Canberra to map barriers and spawning habitat in the Cotter River under various flow conditions. The findings of this project will help inform management actions to ensure that Macquarie perch have access to adequate spawning grounds.

Conclusion

Fyke netting continues to be a reliable technique for detecting Macquarie perch in pools of the Cotter River. Backpack electrofishing, however, proved to be a poor method of obtaining an estimate of abundance of Macquarie perch in the Cotter River and at the reference site, with only a handful of individuals captured over the three years. This technique is still valid at detecting Macquarie perch outside of the pool habitats sampled by fyke netting (i.e. shallow riffles or runs) and should continue to be employed as a complimentary technique of determining distribution.

Recommendations

8.1 To increase the robustness of the sampling regime to detect a true change in the abundance of Macquarie perch upstream and downstream of Vanitys Crossing, the addition of a second riverine reference site is recommended (see recommendation 2.2).

8.2 Backpack electrofishing should continue to be deployed in assessing the abundance and distribution of Macquarie perch upstream and downstream of Vanitys Crossing, but should not be used to compare abundance of Macquarie perch between sites and years as the number of Macquarie perch captured by this method is very low. (This technique is still of use as an accompaniment to fyke netting to determine changes in distribution (ie presence or absence) of Macquarie perch at a site between years).

9) Will macrophyte beds re-establish in the ECD?

Existing macrophyte beds in Cotter Reservoir have been demonstrated to provide important resting habitat for adult Macquarie perch. It is certain that existing macrophyte beds will be drowned by up to 50 m of water once the reservoir has filled. Modelling indicates that the reservoir will remain within 3 m of Full Supply Level for at least 73 percent of the time once the reservoir has filled, potentially allowing new macrophyte beds to establish. Such macrophyte beds could provide important cover habitat for threatened fish.

Sampling design

Are there existing data and an approach that can be followed? No. There is no existing data for the soon to be inundated reach, but historic aerial photography is available for 2003 (post bushfires) and some data on macrophytes in Cotter Reservoir was collected by Roberts (2006) and Katie Ryan (unpublished data). Air photo interpretation is a standard technique for examining vegetation community structure (not floristics), and a rapid survey of macrophyte establishement can be conducted after the ECD fills.

Target species and life stage: emergent macrophyes

Methods: Primary data collection is via aerial photography analysis. Information can be supplemented by visual survey on ground.

Is this method known to be effective?: Yes, on-ground survey and airphoto interpretation are used in standard vegetation assessment

Timing: variable, dependant on whether ad-hoc reporting reveals establishment of macrophyte beds. If macrophyte beds establish then biennial assessment could be conducted.

How long to monitor for?: Macrophytes are not likely to establish until after the ECD fills. As soon as filling has occurred, a variety of ad-hoc reporting mechanisms will be established (e.g. reporting forms issued to field survey teams conducted regular monitoring programs) with regular air photo interpretation to follow, plus a rapid on-ground visual survey .

Number of sites: Dependant on degree of establishment of macrophytes.

Number of replicates: yet to be determined, air photo interpretation would cover entire reservoir perimeter, as would rapid on-ground survey

Information to be collected: emergent macrophyte species, distribution and areal coverage.

Analysis: This is a survey. No pre intervention power analysis required

This question cannot be addressed until the enlarged Cotter Reservoir has reached FSL for an extended period of time.

10) Will translocated Macquarie perch populations survive the initial translocation procedure and reproduce?

Translocation of freshwater fish has been practiced in Australia for more than 100 years, but has a chequered success rate. Recent translocation efforts are more rigorous and structured than historic translocations, but still have often been inadequately monitored to demonstrate success or to investigate how future translocation efforts may be improved. A translocation of adult Macquarie perch in the Queanbeyan River could not be demonstrated to be successful (self sustaining) until more than 10 years after the initial translocation, with survival of translocated fish unable to be ascertained after five years of annual monitoring (Lintermans 2006). Current translocation efforts from Cotter Reservoir commenced in 2006 and involve young-of-year and juvenile fish, and so may require a long lead time before success can be ascertained. Based on the Queanbeyan River experience, failure to detect survival of translocated individuals does not necessarily mean failure, and monitoring may be required for 10-15 years to determine whether a population is self sustaining.

This management question is being addressed in a separate project (see Lintermans 2011).

11) Will Two-spined blackfish establish a reproducing population in the ECD and will they persist in the newly inundated section of the river?

Two-spined blackfish are currently absent from Cotter Reservoir (thought to be a result of excessive sedimentation smothering potential spawning sites) but are present in the river reach to be inundated by the ECD. Newly inundated habitats around the perimeter of the ECD should provide suitable spawning habitats for the species (using Bendora Reservoir as a reference site). Use of Bendora and Corin Reservoirs as reference sites (containing recruiting populations of Two-spined blackfish) will allow determination of whether a lack of establishment or recruitment of Two-spined blackfish in Cotter Reservoir is reservoir specific or a more widespread phenomenon. Similarly, a monitoring program will determine whether the species persists in the newly inundated river reach, and expands to colonise newly inundated habitats around the perimeter of the ECD.

Sampling design

One riverine impact site (Bracks Hole – which will be inundated when the ECD fills), Cotter Reservoir, and two reference reservoirs (Bendora Reservoir and Corin Reservoir) were assessed for Two-spined blackfish presence and evidence of reproduction. Five riverine reference sites on the Cotter River (upstream of the ECD inundation area) also were sampled. These are (in order from most downstream to most upstream); Vanitys Crossing, Spur Hole, Pipeline Road Crossing, Burkes Creek Crossing and Downstream of Bendora Dam. An external reference site (Micalong Creek) also was sampled. Each site was sampled once a year using 12 fyke nets. Fyke nets were set around the shoreline in reservoirs and three per pool for four pools at the riverine sites. The riverine sites were also electrofished with a backpack electrofisher, but the impact site (Bracks Hole) will not be able to be sampled by this method following inundation. Electrofishing occured over 4 x 30 m sections of riffle/run habitat. Sampling occurred in late summer to autumn.

Sampling of the six riverine sites was conducted in summer 2010 (8/2/2010 – 22/2/2010), autumn 2011 (07/03/2011 – 07/04/2011) and summer 2012 (18/01/2012 – 09/02/2012). Micalong Creek could not be sampled in 2012 as a result of heavy rains and flooding, and in 2011 electrofishing could not be conducted at this site as a result of high turbidity and poor visibility. Sampling of the three reservoirs was conducted in autumn 2010 (29/04/2010 – 20/05/2010), 2011 (04/04/2011 – 16/05/2011) and 2012 (12/04/2012 – 02/05/2012).

Results

In 2010, only one Two-spined blackfish captured from the riverine impact site (Bracks Hole, 149 mm TL) by backpack electrofishing, with no Two-spined blackfish captured at this site by fyke nets. In 2010, catches of Two-spined blackfish in fyke nets at the riverine reference sites were highest at Burkes Creek Crossing, though this site had much lower catches in 2011 (Table 16). Of the three reservoirs, Bendora Reservoir had the highest catch of Two-spined blackfish in fyke nets in 2010 (Table 16), with no Two-spined blackfish captured in Cotter Reservoir. Two-spined blackfish have not been recorded from Cotter Reservoir since sampling began in the 1980s (Lintermans 2005a). The relatively low numbers of Two-spined blackfish caught in Corin Reservoir was not expected, as it had been assumed that this reservoir would support similar numbers of Two-spined blackfish to Bendora Reservoir. Low water temperatures might have influenced Two-spined blackfish catchability (11°C in 2010 compared to 15.7°C in 2012). Corin Reservoir has not previously been sampled with fyke nets (Lintermans unpubl data). In 2011 Two-spined blackfish were again absent from Cotter Reservoir, though a small number of individuals (five, ranging in length from 190 – 225 mm TL, all of which are adult) were captured from Bracks Hole (Table 16 & Figure 54).

In 2012, five Two-spined blackfish were captured in fyke nets in Cotter Reservoir ranging in size from 86 – 223 mm TL (Figure 56) and nine were caught from Bracks Hole (fyke nets and backpack electrofishing) ranging in size from 62 – 228 mm TL (Figure 55). The presence of the species in Cotter Reservoir is likely a result of the temporary increase in water level in the reservoir (caused by high rainfall) as a result of the reservoir enlargement program. This has likely facilitated downstream dispersal from inundated upstream environments where the species was known to occur (below Bracks Hole). The catch rate in Bendora Reservoir had dropped from the previous year (Figure 59, Table 16) however the catch rate from Corin Reservoir had increased (Figure 62, Table 16). The CPUE for fyke nets for all riverine sites had decreased for 2012 except for Bracks Hole (impact site) and Burkes Creek Crossing which had an increased catch rate (Table 16).

Table 16. Mean (± SE) CPUE and mean length (TL mm ± SE) of Two-spined blackfish caught by fyke nets in the Cotter River Catchment, and an external reference site (Micalong Ck.) in 2010, 2011 & 2012. CPUE is no. fish per fyke-net hour.

	2010		2011		2012	
	CPUE	Length	CPUE	Length	CPUE	Length
Cotter Reservoir	0	0	0	0	0.02 ± 0.02	128 ± 24
Bracks Hole	0	0	0.02 ± 0.01	203 ± 6	0.03 ± 0.02	168 ± 30
Vanitys Crossing	0.12 ± 0.03	149 ± 12	0.29 ± 0.05	167 ± 4	0.15 ± 0.03	170 ± 5
Spur Hole	0.21 ± 0.03	157 ± 7	0.22 ± 0.04	161 ± 5	0.20 ± 0.03	172 ± 5
Pipeline Rd. Crossing	0.14 ± 0.03	137 ± 10	0.16 ± 0.04	158 ± 6	0.06 ± 0.02	179 ± 7
Burkes Ck. Crossing	0.26 ± 0.06	141 ± 7	0.08 ± 0.03	192 ± 9	0.13 ± 0.03	155 ± 7
D/S of Bendora	0.01 ± 0.01	174 ± 20	0.01 ± 0.01	230	0	131
Bendora Reservoir	0.10 ± 0.07	181 ± 12	0.07 ± 0.02	177 ± 13	0.05 ± 0.02	168 ± 19
Corin Reservoir	0.03 ± 0.01	162 ± 7	0.06 ± 0.02	186 ± 8	0.10 ± 0.03	172 ± 12
Cotter Hut	0.17 ± 0.03	151 ± 6	0.10 ± 0.02	164 ± 5	0.10 ± 0.03	171 ± 7
Micalong Ck.	0.04 ± 0.03	183 ± 13	0.02 ± 0.01	213 ± 13	NS	NS

All riverine sites able to be sampled recorded lower catches of Two-spined blackfish by backpack electrofishing in 2011 compared with 2010, with the biggest variations between years recorded at Spur Hole and Burkes Ck Crossing (Table 17). There was an increase in the CPUE for Bracks Hole (impact site), Spur Hole and Burkes Ck. Crossing for fish caught by backpack electrofishing in 2012 when compared to the previous year, however, the other sites had a decreased catch rate (Table 17).

Table 17. Mean (± SE) CPUE and mean length (TL mm ± SE) of Two-spined blackfish caught by backpack electrofishing (fish per hours of back pack electrofishing) in the Cotter River Catchment, and an external reference site (Micalong Ck.) in 2010, 2011 & 2012. Note: NS = not sampled.

	2010		2011		2012	
	CPUE	Length	CPUE	Length	CPUE	Length
Cotter Reservoir	n/a	n/a	n/a	n/a	n/a	n/a
Bracks Hole	8 ± 8	149	NS	NS	25 ± 17	111 ± 43
Vanitys Crossing	203	95 ± 14	131 ± 35	146 ± 8	57 ± 34	137 ± 18
Spur Hole	214 ± 17	105 ± 10	42 ± 17	140 ± 7	80 ± 29	161 ± 7
Pipeline Rd. Crossing	69 ± 69	78 ± 12	63 ± 14	140 ± 15	36 ± 29	136 ± 32
Burkes Ck. Crossing	112 ± 30	134 ± 13	41 ± 33	152 ± 10	113 ± 23	143 ± 7
D/S of Bendora	10 ± 6	158 ± 13	8 ± 8	115	0	0
Bendora Reservoir	n/a	n/a	n/a	n/a	n/a	n/a
Corin Reservoir	n/a	n/a	n/a	n/a	n/a	n/a
Cotter Hut	49 ± 30	126 ± 19	71 ± 47	149 ± 10	12 ± 7	123 ± 13
Micalong Ck.	93 ± 24	180 ± 7	NS	NS	NS	NS

Based on the length frequency of individuals captured at Bendora Reservoir and Corin Reservoir, 2010, 2011and 2012 were not years with strong YOY recruitment, when compared to Cotter River sites (Figure 57 – Figure 65). Recruitment of Two-spined blackfish at the external reference site was not detected in 2010 or 2011 (Figure 66 & Figure 67).

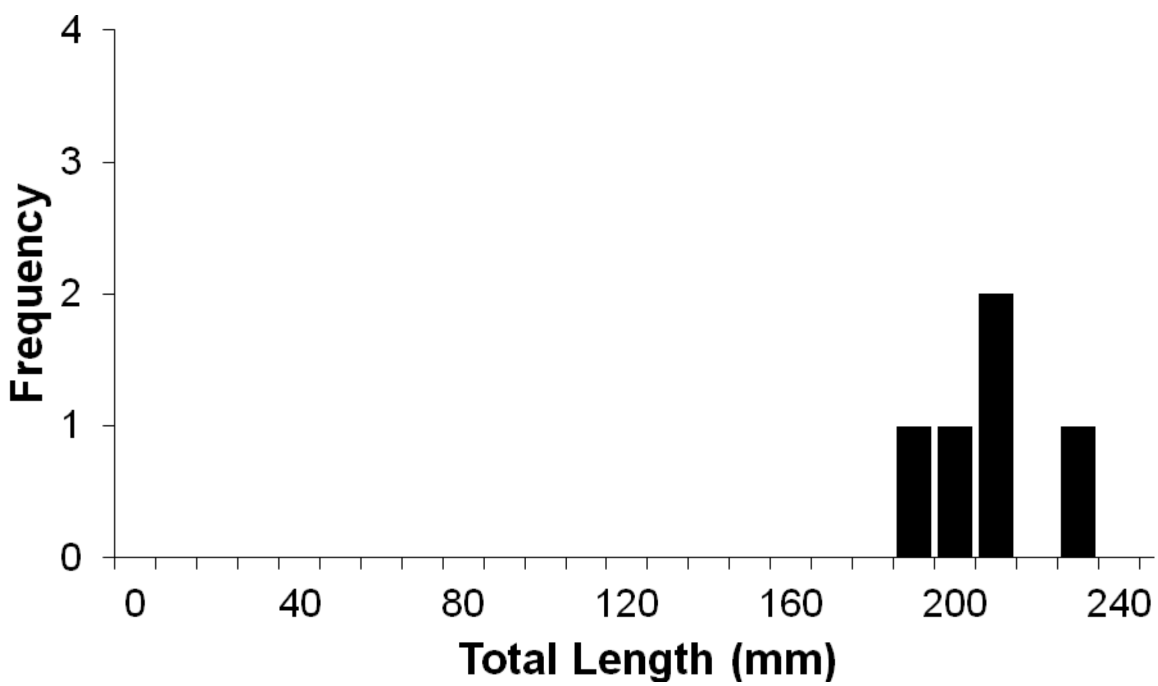


Figure 54. Length of Two-spined blackfish caught from Bracks Hole using fyke nets and backpack electrofishing in March 2011.

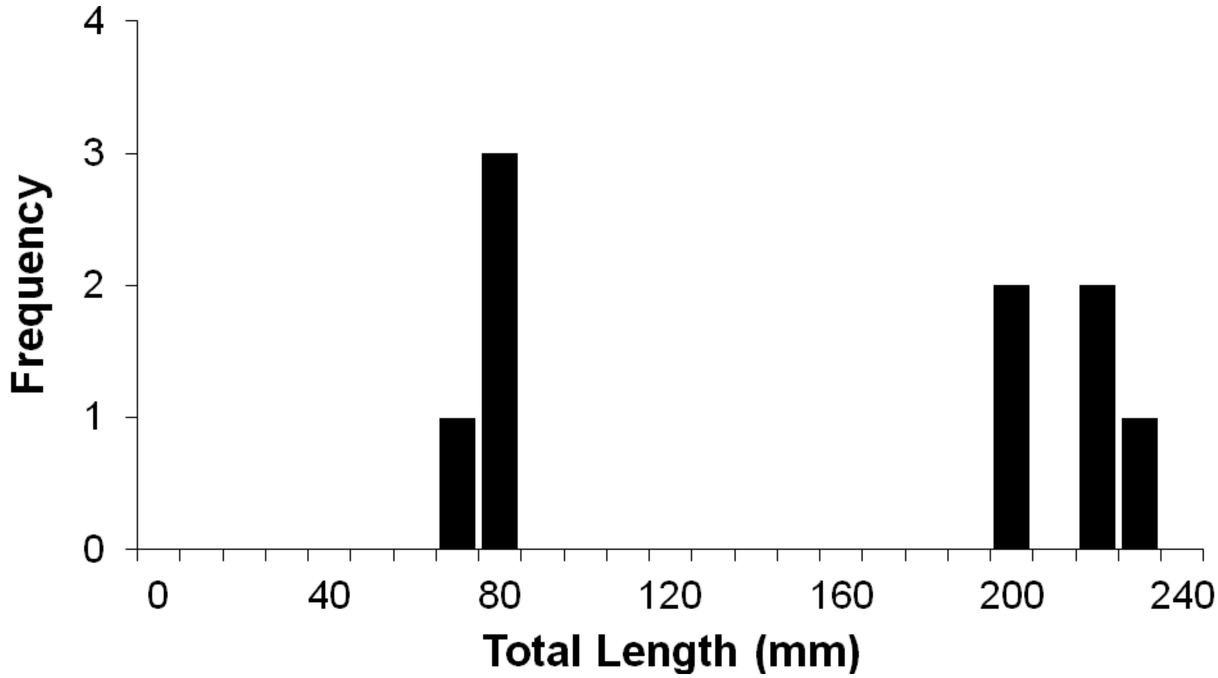


Figure 55. Length of Two-spined blackfish caught from Bracks Hole using fyke nets and backpack electrofishing in February 2012.

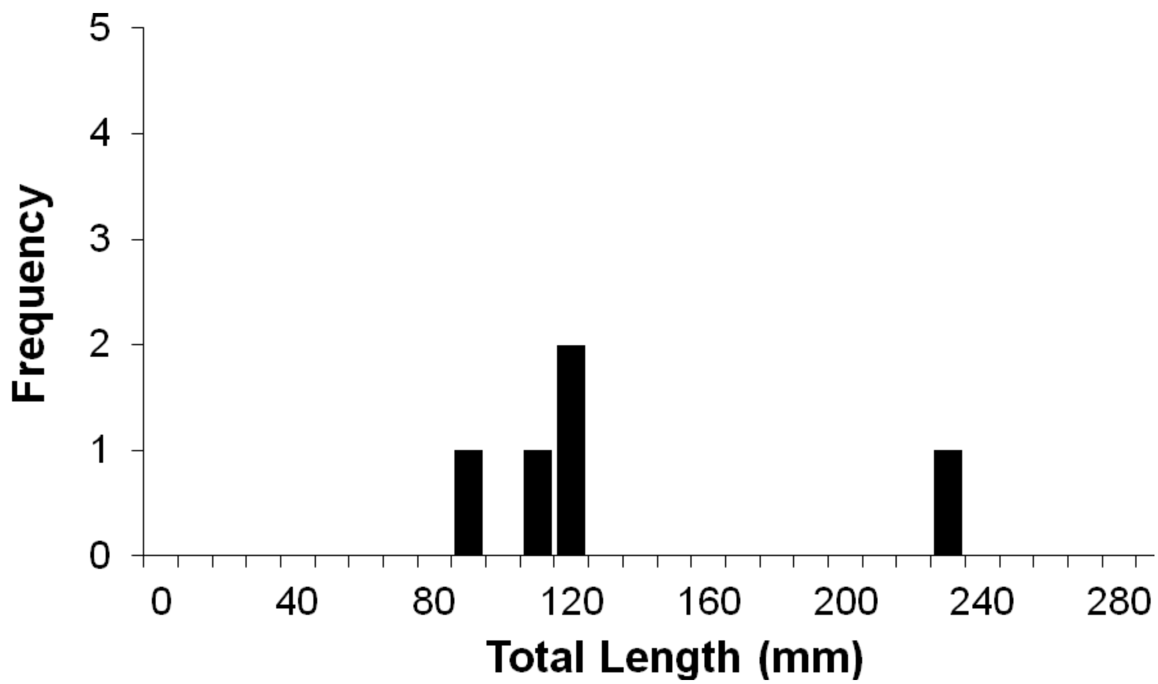


Figure 56. Length of Two-spined blackfish caught from Cotter Reservoir using fyke nets in May 2012.

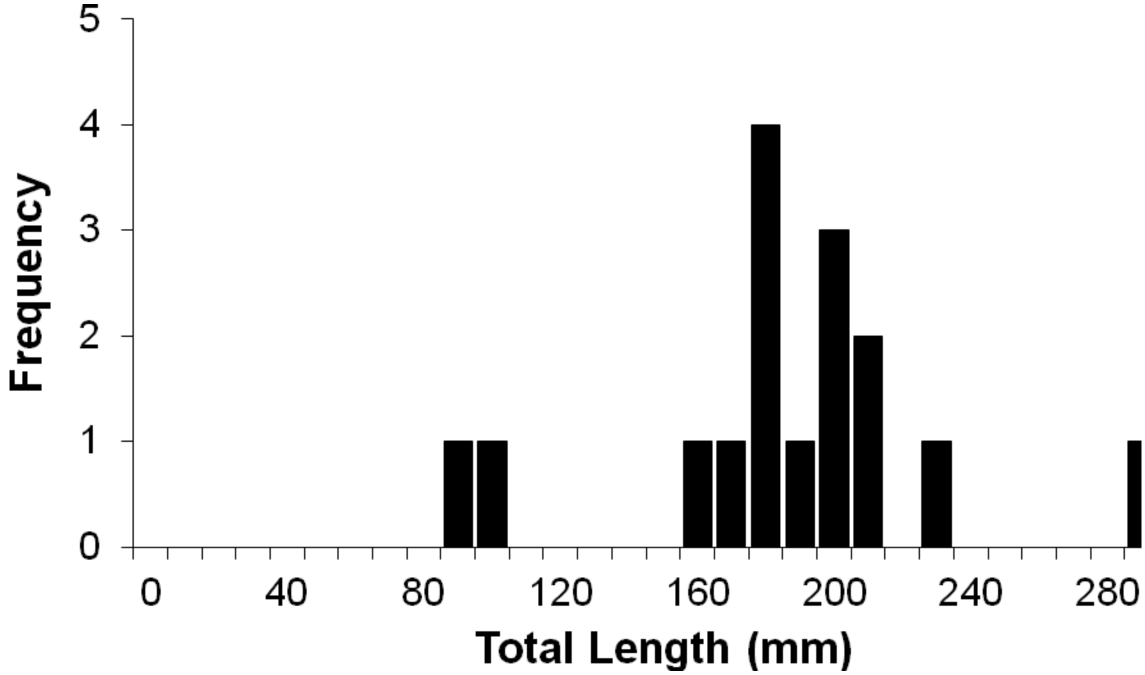


Figure 57. Length of Two-spined blackfish caught from Bendora Reservoir using fyke nets in May 2010.

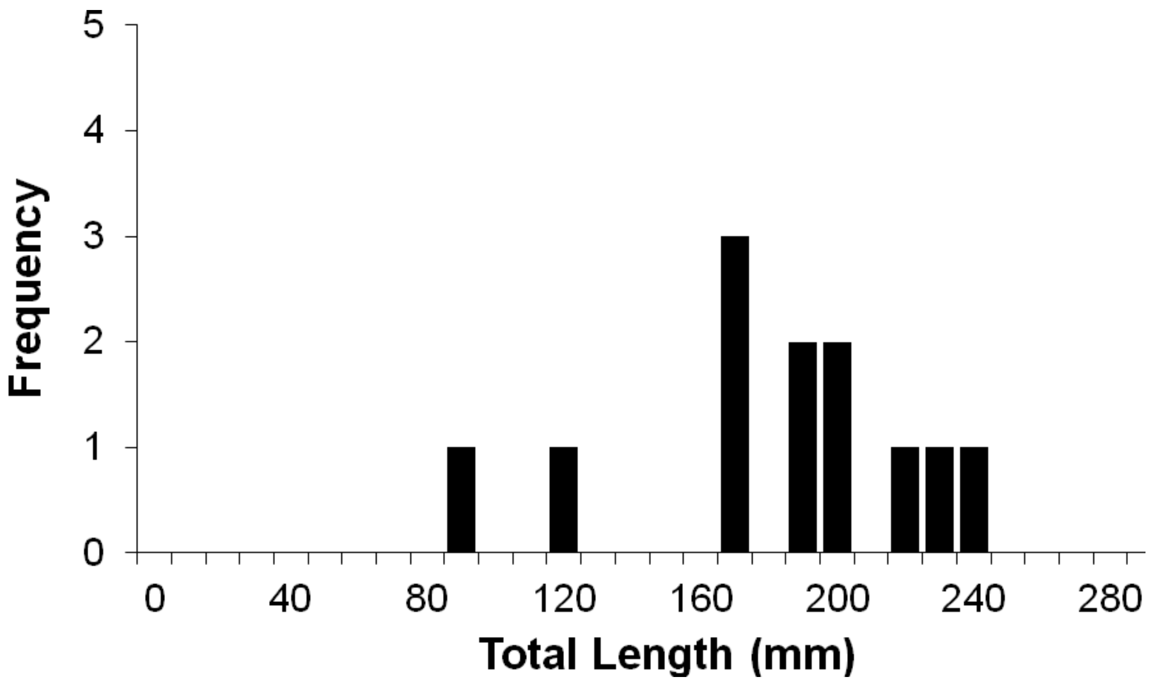


Figure 58. Length of Two-spined blackfish caught from Bendora Reservoir using fyke nets in May 2011.

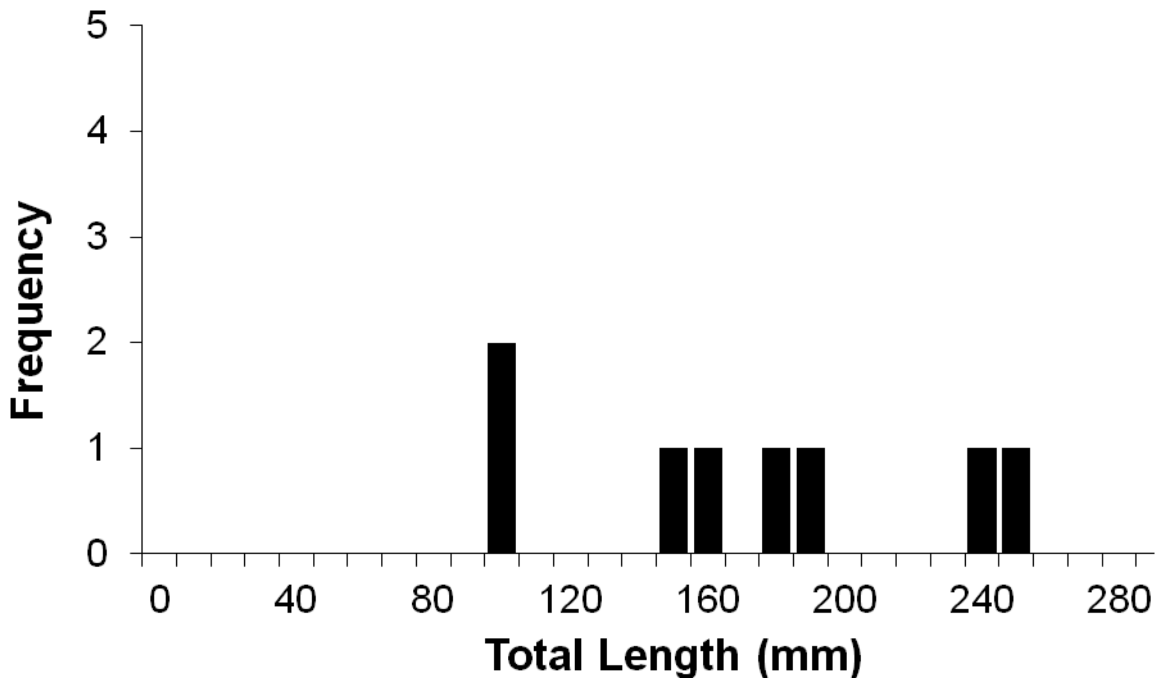


Figure 59. Length of Two-spined blackfish caught from Bendora Reservoir using fyke nets in April 2012.

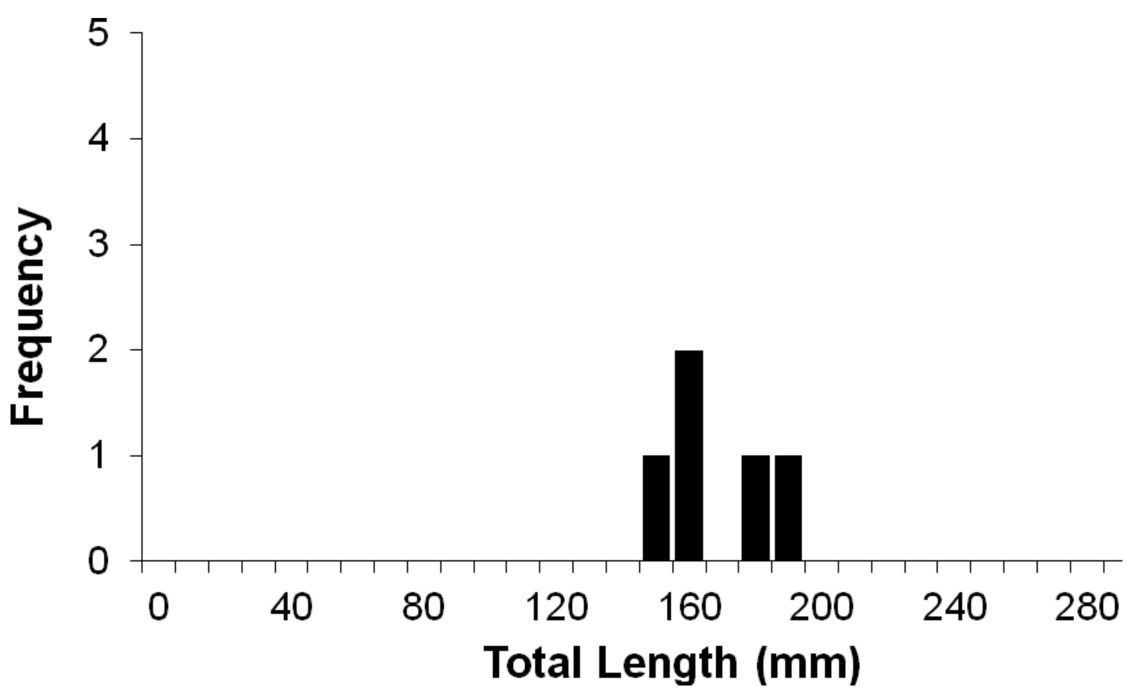


Figure 60. Length of Two-spined blackfish caught from Corin Reservoir using fyke nets in May 2010.

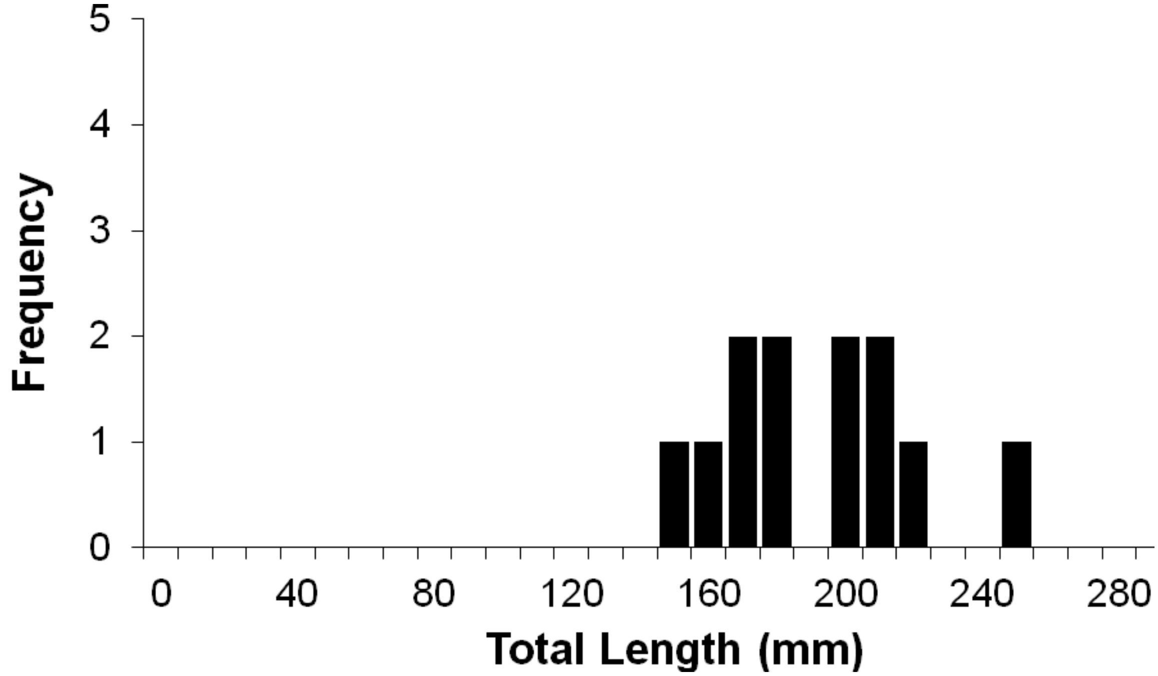


Figure 61. Length of Two-spined blackfish caught from Corin Reservoir using fyke nets in May 2011.

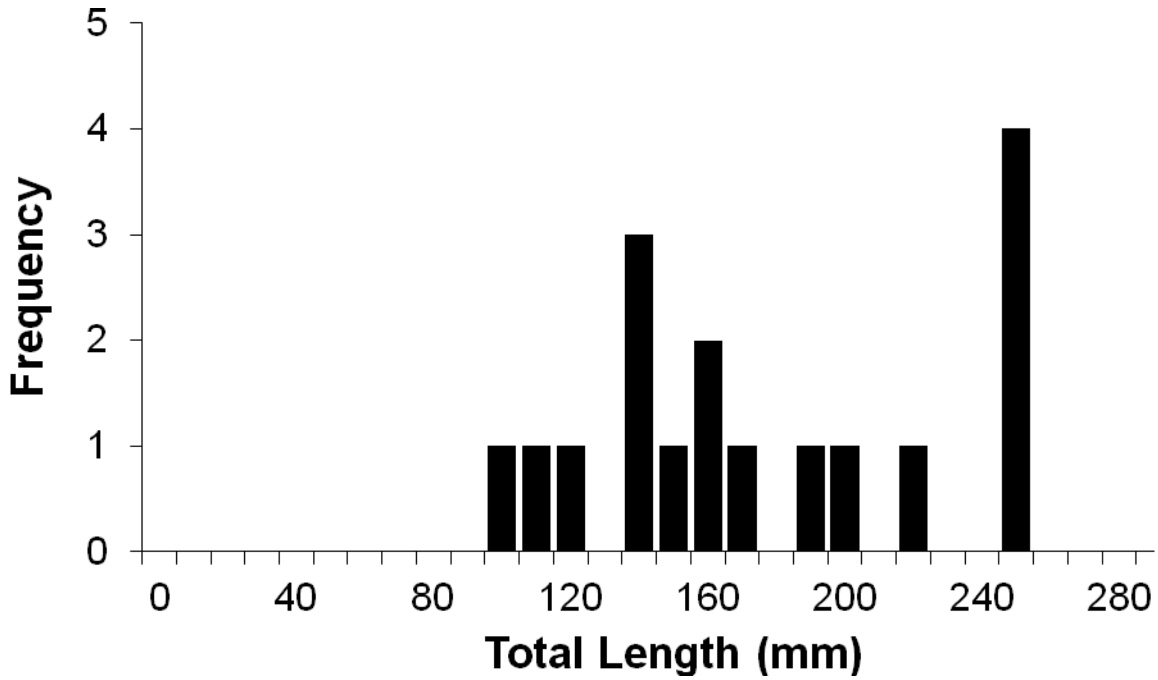


Figure 62. Length of Two-spined blackfish caught from Corin Reservoir using fyke nets in April 2012.

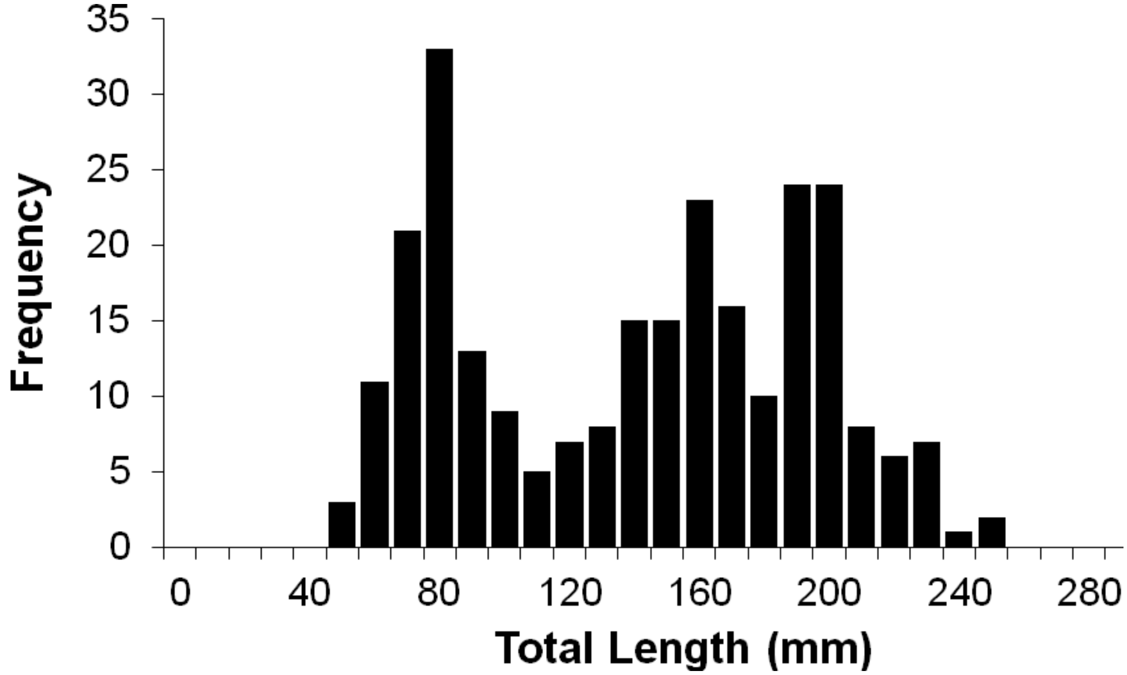


Figure 63. Length of Two-spined blackfish caught by fyke nets and backpack electrofishing from all Cotter River sites in February 2010.

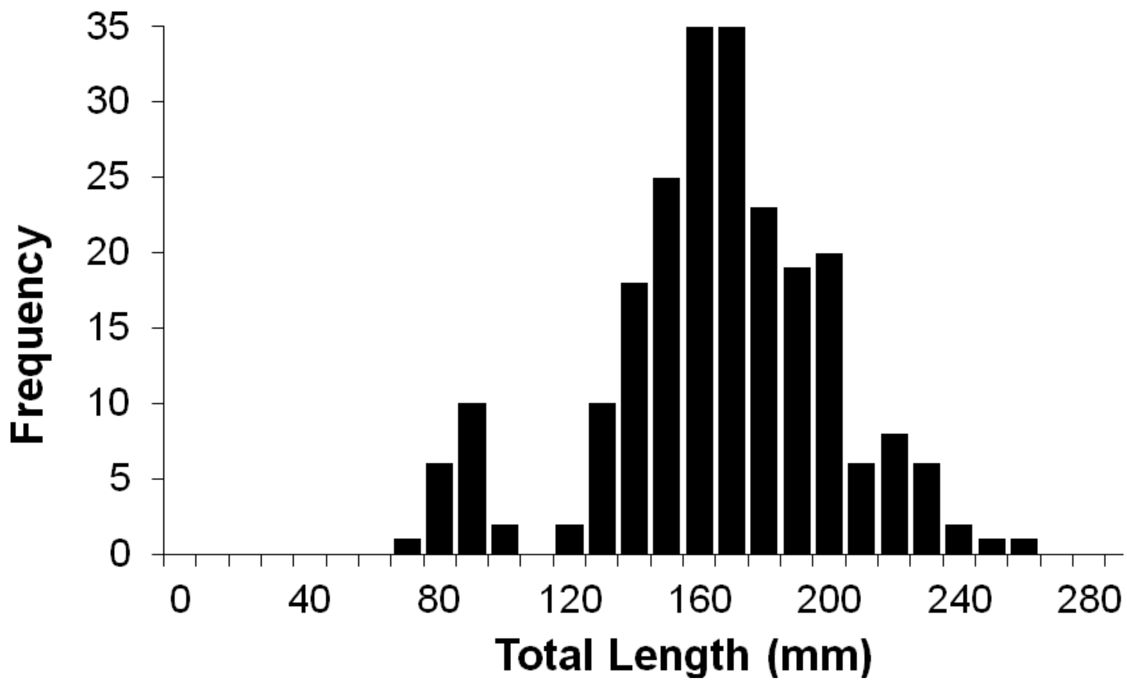


Figure 64. Length of Two-spined blackfish caught by fyke nets and backpack electrofishing from all Cotter River sites in March-April 2011.

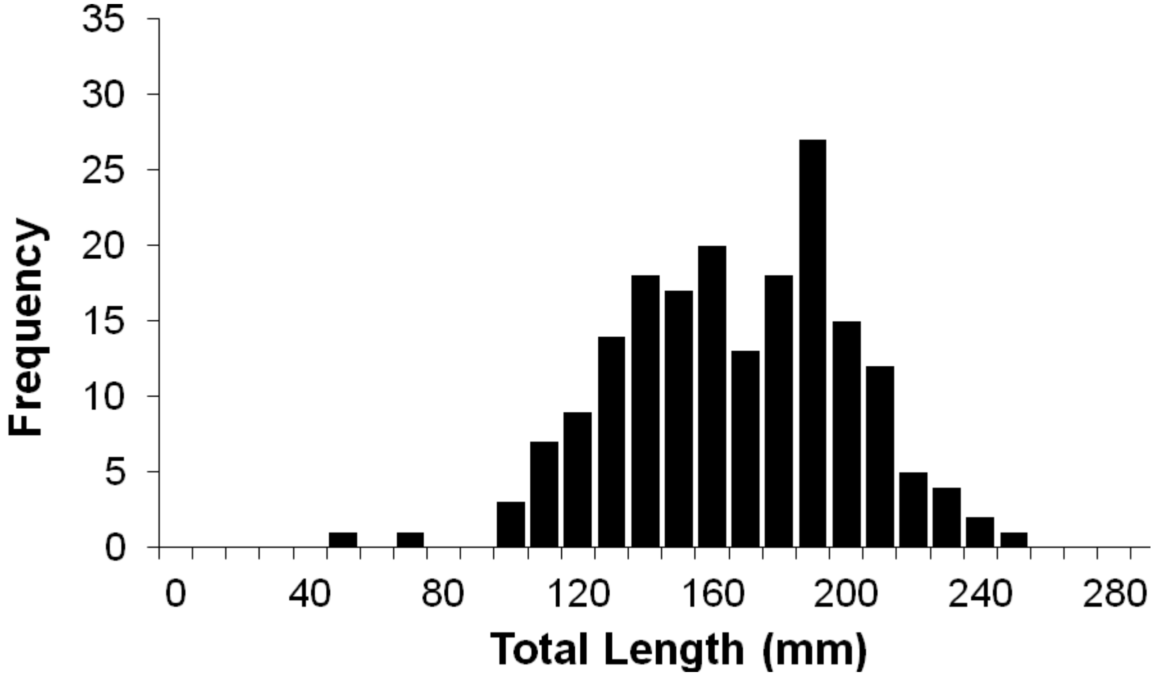


Figure 65. Length of Two-spined blackfish caught by fyke nets and backpack electrofishing from all Cotter River sites in February 2012.

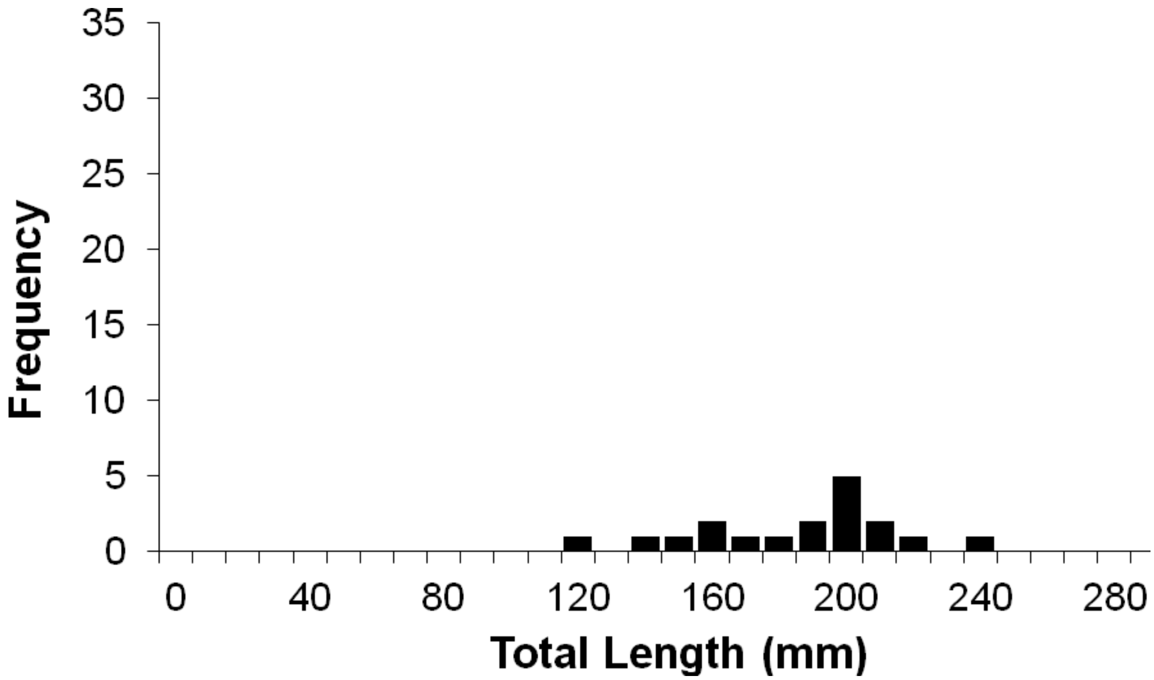


Figure 66. Length of Two-spined blackfish caught by fyke nets and backpack electrofishing from the Micalong Creek reference site in March 2010.

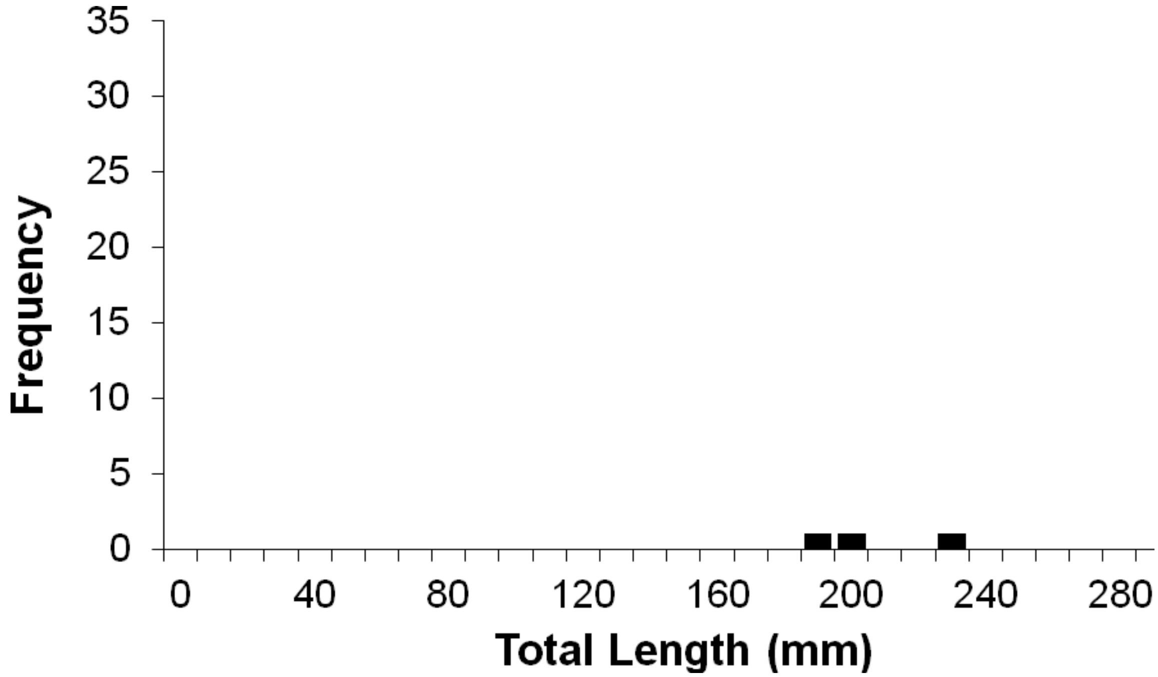


Figure 67. Length of Two-spined blackfish caught by fyke nets from the Micalong Creek reference site in April 2011. NB. No backpack electrofishing conducted because of high flows and high turbidity.

Discussion

Whilst this question cannot be answered until the ECD has filled, we can make comment on the current state of the Two-spined blackfish population in the current Cotter Reservoir, the inundation zone and the Cotter River elsewhere.

Two-spined blackfish have not been detected in the Cotter Reservoir for many years but is present in both Bendora and Corin Reservoirs (Lintermans, unpublished data; Lintermans 2005a; Ebner and Lintermans 2007; Ebner *et al.* 2008). It is believed that sedimentation associated with forestry activities has destroyed suitable breeding habitat in the Cotter Reservoir and prevented re-colonisation by this species. Results from the 2010 and 2011 fyke netting survey of the Cotter Reservoir supported earlier findings of this species' absence from Cotter Reservoir; however, in 2012 five Two-spined blackfish were captured in its waters. Four were collected together at a freshly placed rock pile (leftovers from the rock reef construction for the study by Lintermans *et al.* (2010) and one from just upstream. It is uncertain whether these individuals were temporarily displaced by the 2011 floods and will eventually make their way back to the river or whether these individuals had colonised newly formed rock habitat during the March 2012 rise in reservoir water level, or possibly a combination of both displacement and colonisation. In any event, the use of the newly placed rock by Two-spined blackfish bodes well for the potential of this species using the constructed rock reeks deployed around the enlarged reservoir which were primarily aimed at adult Macquarie perch.

Two-spined blackfish were found to be in low abundance in the river immediately upstream of Cotter Reservoir when compared to other sites upstream. This is consistent with the findings of Ebner *et al.* (2008) who also found that the site immediately upstream of Cotter Reservoir had lower abundances of Two-spined blackfish compared to sites upstream. Surveys conducted in 2001 and 2002 (Ebner and Lintermans 2007) reported higher abundances of Two-spined blackfish in the river immediately upstream of Cotter Reservoir, compared to the current study and that of Ebner *et al.* (2008). It is thought that sedimentation associated with the 2003 bushfire (and subsequent heavy rains which washed large amounts of sediment into the Cotter River (Carey *et al.* 2003) may have decreased the suitability of the Cotter River upstream of Cotter Reservoir for Two-spined blackfish and that environmental flow releases were insufficient to remediate this site. The current study also found Two-spined blackfish to be at very low abundances at the site immediately downstream of Bendora Dam. Ebner *et al.* (2008) also reported low abundances of Two-spined blackfish at this site and cited the large abundance of trout present as a possible cause by way of predation and competition for food. Indeed, the site downstream of Bendora Dam was found to have the highest abundances of trout in the current study which suggests a negative correlation between trout abundance and Two-spined blackfish abundance. Whether this is a causal relationship or due to a secondary factor is not clear. Monitoring programs for of environmental flows in the Cotter River have also noted the low abundance of Two-spined blackfish at this site (Lintermans 2005b; Clear *et al.* 2007) with poor habitat and water quality noted by (Lintermans 2005b).

Conclusion

The current study design appears adequate to determine if Two-spined blackfish establish a reproducing population in the ECD and if they will persist in the newly inundated section of the river

Recommendations

11.1 The current sampling regime should continue to assess whether Two-spined blackfish will establish a reproducing population in the ECD and will they persist in the newly inundated section of the river.

12) Will there be significant changes in the abundance and distribution of Goldfish and Oriental weatherloach in the ECD?

Goldfish and Oriental weatherloach are currently present in Cotter Reservoir and have noted preferences for still-water habitats. The enlargement of the reservoir will provide a significant increase in habitat for these species, and a consequent increase in abundance could occur. These species could competitively interact with Macquarie perch for resources (particularly food and shelter), but are not a predatory threat. Also, expansion of populations of Goldfish and Oriental weatherloach could facilitate the expansion of trout populations, which are a potential predation threat to threatened fish populations. Both species have been recorded in trout diet from the reservoir, with goldfish being particularly dominant. Monitoring of changes in status of Goldfish and Oriental weatherloach in the reservoir, along with monitoring of trout diet (Question 3) will provide insights into the dynamics of the fish community in the reservoir.

Sampling design

Annual Goldfish and Oriental weatherloach abundance and distribution was determined for Cotter Reservoir and two reference sites (Kissops Flat on the upper Murrumbidgee River and Lake Ginninderra on Ginninderra Creek). At each site 12 fyke nets were set overnight. Sampling of Kissops Flat occurred on 20/04/2010, 05/05/2011 and 16/04/2012. Sampling of Cotter Reservoir (04/05/2010, 04/04/2011, 02/05/2012) and Lake Ginninderra (23/03/2010, 30/03/2011, 31/01/2012) also occurred in autumn in most years.

Results

A total of four Goldfish and one Oriental weatherloach were caught from Cotter Reservoir in 2010. The Goldfish were 58, 84 and 112 mm FL and the Oriental weatherloach was 134 mm TL. The fourth Goldfish was not able to be accurately measured as it had been damaged by a water rat. It is possible that low water temperatures (~15°C) at the time of sampling of Cotter Reservoir in 2010 may have influenced the low catch rate of Goldfish. In 2011 a total of 38 Goldfish were caught in Cotter Reservoir ranging in size from 55 – 160 mm FL (Figure 68). Only one Oriental weatherloach was caught measuring 147 mm TL. In 2012, only a single Goldfish was sampled from Cotter Reservoir measuring 118 mm FL and two Oriental weatherloach measuring 96 and 91 mm TL.

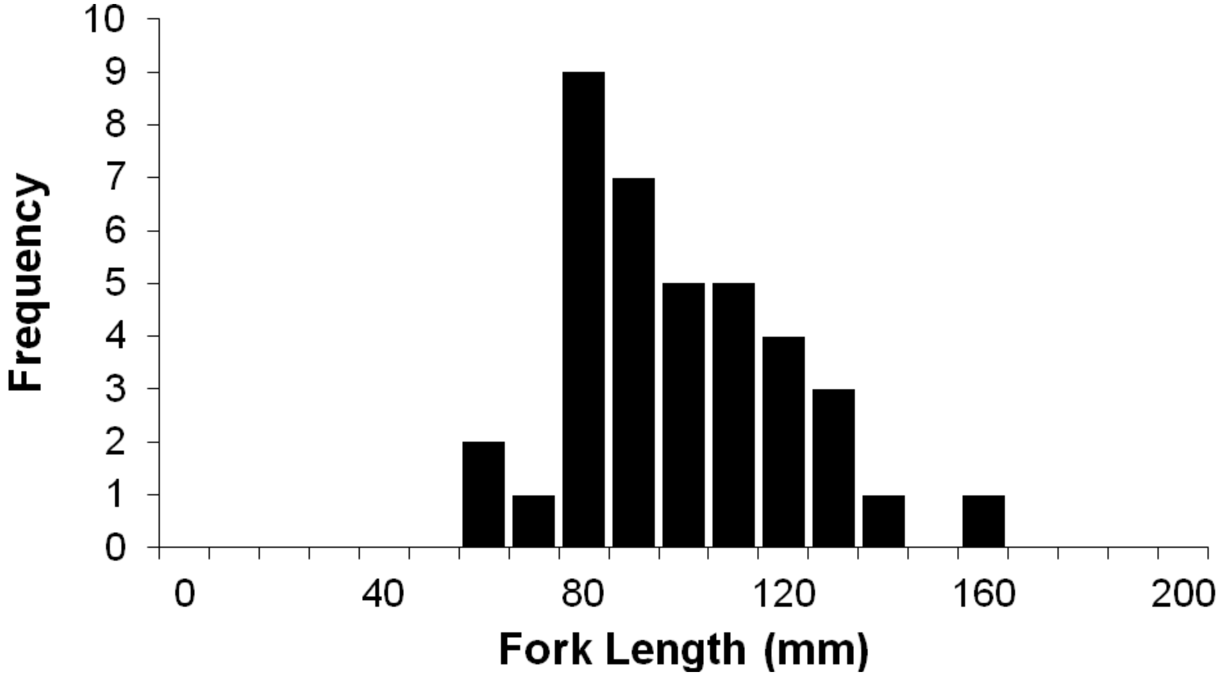


Figure 68. Length of Goldfish caught using fyke nets in Cotter Reservoir in April 2011.

In 2010 a total of nine Goldfish were caught at the riverine reference site, Kissops Flat, ranging in size from 48 – 85 mm FL (Figure 69). In 2011 only two Goldfish were caught (80 & 82 mm FL). In 2012 six Goldfish were captured at Kissops Flat ranging in size from 70 – 113 mm FL (Figure 70).

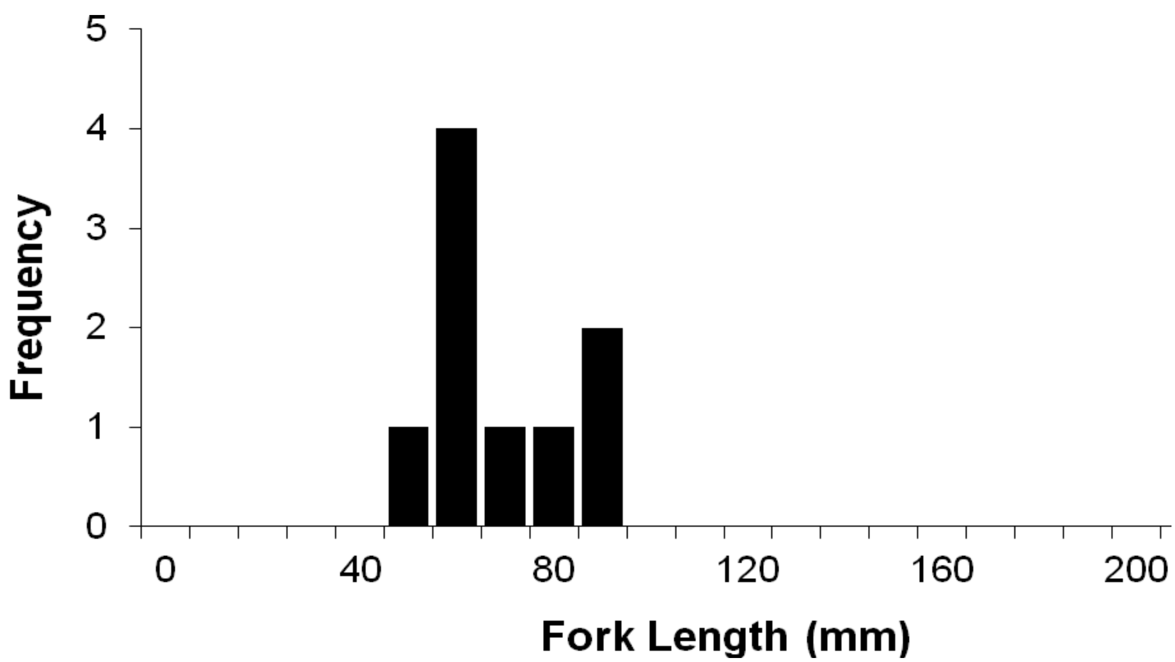


Figure 69. Goldfish caught using fyke nets at Kissops Flat in April 2010.

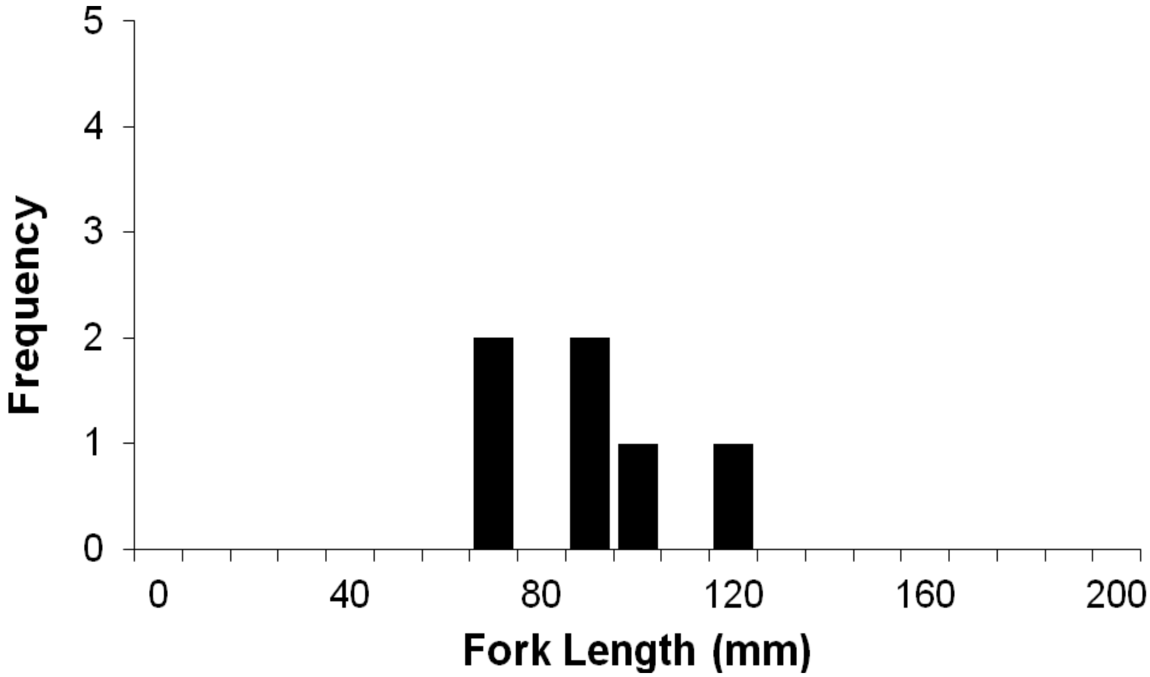


Figure 70. Goldfish caught using fyke nets at Kissops Flat in April 2012.

No Goldfish or Oriental weatherloach were captured in any of the samplings at the lacustrine reference site, Lake Ginninderra.

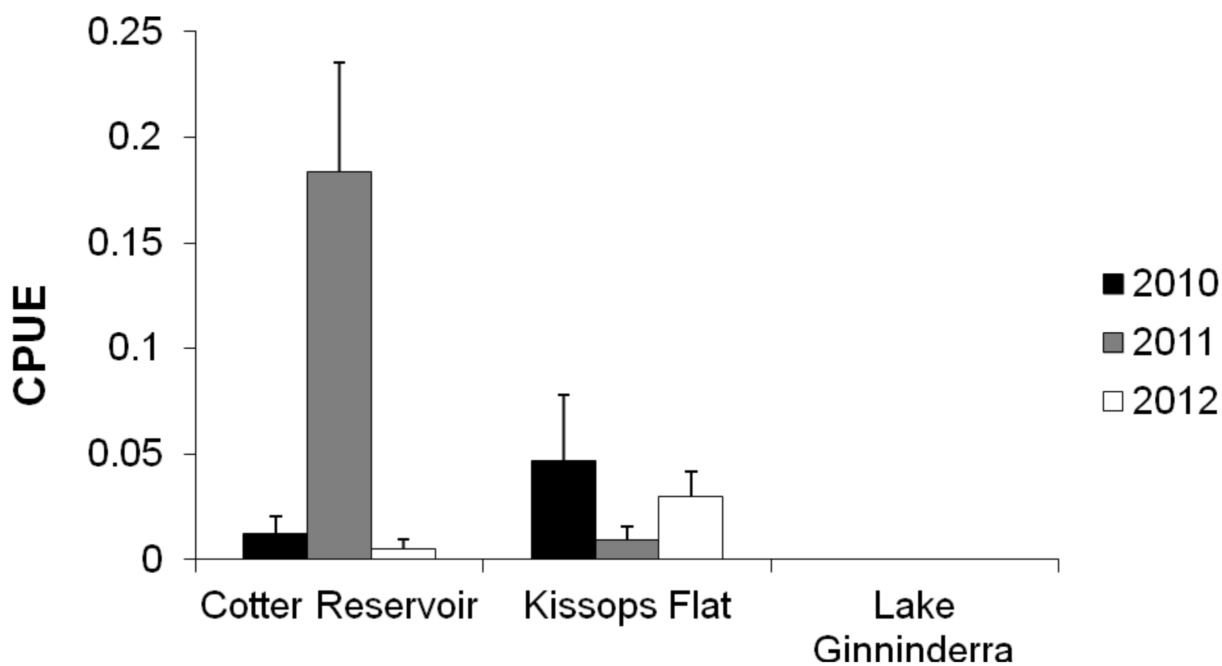


Figure 71. Mean (± SE) Goldfish captured per fyke-net hour (CPUE).

Discussion

There was large variation in the abundance of Goldfish in the Cotter Reservoir between years. The abundance of Goldfish in 2011 was eight-fold that of the other two years. The numbers of Goldfish caught in each net were relatively evenly spread, discounting that a one-off large catch of a school of goldfish can explain the increased abundance in 2011. Increases in Goldfish abundance between years in the Upper Mississippi River, USA, were found to be associated with low flow and elevated water temperatures (Day *et al.* 1996). It is unlikely that these factors were responsible for the increased abundance of Goldfish in Cotter Reservoir in 2011 as flow is minimal in an impoundment, and water temperatures would be more stable than in a riverine environment. However, although sampling was conducted in Autumn in all years, sampling in 2011 was conducted one month earlier than in 2010 and 2012, and so water temperature was 3-4 degrees warmer (15.8 °C in 2010, 20.1°C in 2011, 16.8 °C in 2012), and this may have affected capture rates. Sampling date has been suggested as influencing catch rates of another cyprinid in a Canberra lake (Carp in Lake Burley Griffin)(Lintermans 1995; Lintermans 1996) and this may be the case with Goldfish in the ECD. Earlier sampling (April) for Goldfish in the ECD is recommended for future years. The water level in the Cotter Reservoir was actually higher than or roughly equal to FSL during the time of sampling and for the preceding few months, and potentially this inundation of new ground prompted a release of nutrients facilitating additional spawning or improved growth or survival of juvenile Goldfish. Goldfish generally breed in summer at water temperatures of 17-23 °C (Lintermans 2007) and can grow quickly when conditions are suitable. The majority of individuals captured in 2011 were less than six months of age based on another Australian study (Morgan and Beatty 2007) suggesting that there had been a large recruitment event in late 2010 or early 2011. Abundances of Goldfish at the riverine reference site (Kissops Flat) were relatively consistent over the three years, with a slight drop in abundance in 2011.

Only a small number (four) of Oriental weatherloach were captured over the three years of sampling, comprising of one in 2010, one in 2011 and two in 2012. Such low abundance is common for Cotter Reservoir based on previous fyke net surveys (Broadhurst, unpublished data). How the population of Oriental weatherloach will respond to the filling of the ECD remains uncertain.

Conclusion

No Goldfish or Oriental weatherloach were captured at the reference impoundment (Lake Ginninderra) over the three years of sampling. These species are known to be present in the impoundment, but at present must be at a density too sparse for detection. The use of a second or alternative impoundment to provide a reference for the populations of Goldfish and Oriental weatherloach should be explored.

Recommendations

12.1 The use of a second reference impoundment should be explored that has comparable abundances of Goldfish and Oriental weatherloach to Cotter Reservoir (Yerrabi Pond, Gungahlin, is the suggested choice). To date, sampling of the current reference site has failed to capture either of these alien species and therefore is a poor reference site.

12.2 Sampling for Goldfish and Oriental weatherloach should be conducted in late March/early April in future years before water temperatures drop. Once water temperatures drop, these species are less active and are therefore less likely to be captured by passive gear types like fyke nets (the method used in this study).

MANAGEMENT THRESHOLDS

Develop specific thresholds for management intervention relevant to the 12 management questions:

The EPBC conditions of approval require the identification of thresholds for management intervention in relation to all measures implemented to manage and maintain a viable Macquarie perch population in the Cotter River catchment. As fish abundance and recruitment naturally varies between years, the thresholds are likely to encompass both spatial and temporal factors (i.e. the thresholds will likely have a defined fish abundance trigger as well as what proportion of years such a trigger level must be reached). There are a variety of options for developing specific thresholds to link science and management interventions and these are still being explored. For example Kruger National Park in South Africa and Kosciusko National Park in Australia operate Strategic Adaptive Management systems that utilise Thresholds of Potential Concern (Biggs and Rogers 2003; Foxcroft and Downey 2008). For the ECD, expert opinion was initially proposed as an initial method for developing interim thresholds, with an adaptive management approach then being utilised to refine such thresholds in the light of the data collected during the 'before' monitoring phase. It was decided by the project team (in consultation with the Manager of the ECD Fish Management Program) that it would be more beneficial to explore the data collected in the phase 1 assessment as the basis for establishing the thresholds, and this development will occur in 2013-14. Interim thresholds have already been developed for some elements of the program, with filling phase thresholds in the ECD developed for water quality parameters such as dissolved oxygen that could potentially impact the reservoir Macquarie perch population. Thresholds of cormorant abundance have also developed, along with the appropriate management responses (ACTEW Corporation 2012). It is expected that additional interim thresholds for other population parameters will be established in 2013-14, with refinement of the thresholds occurring over time as additional data is collected, and as the reservoir passes through natural maturation phases (e.g. filling, early post filling, etc).

CONCLUSIONS

After three years the baseline assessment of the potential impacts on threatened fish by the ECD is proceeding well. To date, the baseline assessment has covered both dry (early-mid 2010), wet (late 2010 and 2011) and average (2012) periods, when compared to recent history. The field assessment techniques are generally performing well. However, the commencement of the filling phase of the ECD will no doubt affect capture of adult Macquarie perch in gill nets and investigation of an alternative technique is required for assessing adult Macquarie perch abundance in a filling reservoir. The project team are well placed to trial boat electrofishing as this alternative technique. Further development of genetic techniques for determining trout predation on Macquarie perch is required if this approach is to meet the detection expectations of the monitoring program. Additional funding for further genetic work will be sought, separately to the ECD fish monitoring program. The approach of minimising field sampling costs and duplication by answering multiple management questions simultaneously has proved effective, with the exception of the spring diet sampling for trout.

The selection of reference sites in the sampling program has generally been appropriate and beneficial, with two changes recommended. The addition of another site in the upper Murrumbidgee catchment (additional to Kissops Flat) will provide a more robust measure of the status of this Macquarie perch population. The Lake Ginninderra reference site for alien fish abundance (Goldfish, Oriental weatherloach) in Cotter Reservoir has performed poorly, with very few captures of these target species, and another reservoir reference site for these species should be explored.

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APPENDIX A

DNA-based identification of larval Macquarie perch (*Macquaria australasica*) and two-spined blackfish (*Gadopsis bispinosus*) from stomach contents of alien trout in the Cotter Reservoir: report on primer development

Anna MacDonald, Matthew Young and Stephen Sarre
Institute for Applied Ecology, University of Canberra, ACT 2601

Executive Summary

• Macquarie perch (*Macquaria australasica*) is an endangered Australian native fish. A remnant population of this species is found in the ACT, in and upstream of the Cotter Reservoir. Several alien fish species, including predatory trout, are present in this area. At present it is not known whether trout predation poses a threat to the conservation of this Macquarie perch population, or to other native fish in the Cotter, including two-spined blackfish (*Gadopsis bispinosus*), listed as vulnerable in the ACT. In particular, predation by trout of larval fish is difficult to detect because these are rapidly digested in the trout gastro-intestinal tract.

• DNA methods provide an opportunity to improve detection of trout predation on fish larvae, through genetic analysis of trout stomach contents. A genetic approach would remove the need to detect intact, undigested Macquarie perch larvae to confirm the occurrence of predation. To apply DNA detection to this question, PCR primers need to be designed to specifically amplify DNA from the target species of interest, but not from any other species potentially present in the same locality.

• We designed PCR primers to specifically amplify DNA from two native fish species of conservation interest: *M. australasica* and *G. bispinosus*. We also designed primers to specifically amplify DNA from the introduced goldfish *C. auratus*: these primers will be used as a control for detection of prey DNA from trout stomach contents as this species is known to be commonly predated by trout in the Cotter Reservoir. Finally, we designed a set of "universal" fish primers to amplify DNA from all fish species occurring in the Cotter Reservoir. These universal primers will be used as a control for DNA quality in test samples, because DNA from stomach contents is expected to be degraded.

• All four sets of PCR primers were tested on DNA extracted from tissue samples from nine fish species, including all fish present in the Cotter River upstream of Cotter dam, under a range of PCR conditions to determine their specificity to their respective target species.

• PCR conditions for the *G. bispinosus* and *C. auratus* primers were not able to be optimised during the scope of this project: these primers amplified DNA from several non-target fish species. Additional work is needed to test these primers under a wider range of PCR conditions to improve their specificity.

• PCR conditions for the *M. australasica* primers were optimised such that, under the appropriate PCR conditions, DNA from the target species was amplified but no amplification was detected from any of the eight non-target species. The universal fish primers successfully amplified DNA from all nine fish species under all PCR conditions tested.

• Before this DNA test can be applied to investigate predation of *M. australasica* in the Cotter Reservoir and River, further work is needed to test the specificity and sensitivity of the *M. australasica* primers and

the universal fish primers during the analysis of DNA from trout stomach contents. Specific needs include: optimising DNA extraction methods from samples of this type; DNA testing of trout stomach contents known to be positive and negative for *M. australasica* DNA; and testing the sensitivity of the primers through captive feeding trials where trout are fed known quantities of *M. australasica* larvae before DNA testing.

Introduction

Macquarie perch (*Macquaria australasica*) is an Australian native fish that was once widespread in the waterways of the southeast Murray-Darling Basin (Lintermans, 2002, 2007). It is listed as endangered nationally under the Environment Protection and Biodiversity Conservation (EPBC) Act 1999, and locally under the ACT Nature Conservation (NCA) Act 1980 (Lintermans, 2012). There are two morphologically distinct and geographically isolated forms of *M. australasica*, which are considered likely to be separate species (Faulks *et al*., 2010). In the Australian Capital Territory (ACT), a remnant population of the western form is now limited to the Cotter Reservoir and approximately 15 km of river upstream, below Bendora dam (Lintermans 2002; Broadhurst *et al*. in press).

The fish assemblage of the Cotter Reservoir consists of both native and alien fish species. In addition to *M. australasica*, there are two other threatened native fish present in the Cotter Reservoir and river system above the Cotter dam; the two-spined blackfish (*Gadopsis bispinosus*) and the trout cod (*Maccullochella macquariensis*) (Lintermans, 2012). Alien species in the Cotter River above Cotter dam include eastern gambusia (*Gambusia holbrooki*), goldfish (*Carassius auratus*), oriental weatherloach (*Misgurnus anguillicaudatus*), brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) (Lintermans, 2002). Salmonids are piscivorous, and are known to predate upon and influence the assemblages of native Australian fish (Lintermans, 2000). It is currently not known whether trout predation is occurring on the larval stages of *M. australasica* in the Cotter catchment, or how predation could be impacting upon recruitment and survival of this remnant population. A better understanding of the impact of trout upon *G. bispinosus* is also important to conservation of this species. Cotter Reservoir is currently being enlarged with the new reservoir to be 50 m deeper and almost 20 times the capacity of the existing reservoir. The size of individual trout and of the trout population is expected to increase in the enlarged reservoir as a result of increased food supplies associated with trophic upsurge (Lintermans 2012). This enhanced trout population is also likely to have impacts on the aquatic fauna of the inflowing Cotter River, as trout migrate up the river to spawn (Lintermans 2012).

Morphological prey identification methods have been employed to visually identify prey items from the stomach contents of trout from the Cotter Reservoir (e.g. Ebner *et al*., 2007). These methods were successful in identifying goldfish as the main fish species predated upon in this system however they were unsuccessful in identifying whether *M. australasica* were in the diet, and this was postulated as a potential false negative (Ebner *et al*., 2007). Morphological identification of diagnostic features such as pigmentation patterns (Fahay, 1983) on prey species in stomach contents is usually hindered by digestive damage, an issue that is compounded when identifying larval life-stages, which can degrade quickly (Rosel and Kocher, 2002). While morphologically identifiable features may be damaged through the digestive process, molecular DNA-based approaches to prey identification from the partially digested remains in stomach and faecal samples have been successfully adapted for fish (e.g. Rosel and Kocher, 2002) and vertebrate predators (e.g. Jarman *et al*., 2002). In fish, these DNA-based methods have been able to detect the presence of larval fish in stomach/intestinal samples up to 12 hours after initial ingestion by the fish predator (Rosel and Kocher, 2002).

The aim of this study was to develop species-specific DNA tests for *M. australasica* and *G. bispinosus* to be used in future work to determine whether trout (*S. trutta* and *O. mykiss*) are predating upon Macquarie perch (*M. australasica*) larvae within the Cotter Reservoir and upstream of the Cotter Dam using DNA-based methods.

Methods

Primer design

12S and 18S rDNA gene sequences from a range of fish species occurring in the Murray-Darling Basin (Hardy *et al*. 2011) were aligned to identify regions that were diagnostic at the species level, to enable the design of species-specific PCR primers. The intended application of the primers developed is to detect species-specific DNA from trout stomach contents, from which much of the DNA present can be expected to be degraded. Consequently we targeted primers that amplify short DNA fragments (<300 bp) to improve the likelihood of amplification success from degraded DNA samples. Species-specific primers were developed from the 12S gene for three species (Table 1): *M. australasica* and *G. bispinosus*, both of which are threatened native species considered at risk of trout predation, and the introduced goldfish *C. auratus*. The goldfish is known to be a common prey item for trout, so goldfish-specific primers were designed to serve as a control test for amplification success from trout stomach contents.

Universal fish primers were also developed from a region of conserved DNA sequence in the 18S gene, to enable amplification of DNA from potentially any fish species present in a sample. These universal primers were designed to serve as a positive control for DNA amplification success from degraded samples. For example, if these universal primers were used in conjunction with an *M. australasica*-specific set of primers, three results would be possible:

i) no amplification from either set of primers, indicating poor DNA quality,
ii) amplification from the universal primers alone, indicating good DNA quality but no detection of *M. australasica,*
iii) amplification from both the universal primers and the *M. australasica* primers, indicating successful detection of *M. australasica.*

Table 1. Species-specific and universal fish primers designed in this study.

Species	Gene	Primer name	Primer Sequence (5′ to 3′)	Fragment size (bp)
Macquaria australasica	12S	Mac_aus_12S_F22	GTATAATACACCTACTATCCGCCT	205
	12S	Mac_aus_12S_R226	CTGTGCCAATTCTGCTTACTATTAGT	
Gadopsis bispinosus	12S	Gad_bis_12S_F193	CATGAGAGACTTATAGTAAGCAAAACTGGTAC	118
	12S	Gad_bis_12S_R310	GAACATCATTCGTATTCCTTAATTCAAG	
Carassius auratus	12S	Car_aur_12S_F9	GTAAACTTAGACATCCAACTACAATAGA	219
	12S	Car_aur_12S_R227	TTGTACCCATTTTGCTTACTTTTATT	
Universal Fish	18S	Fish_18S_1F	GAATCAGGGTTCGATTCC	
	18S	Fish_18S_3R	CAACTACGAGCTTTTTAACTGC	271

Tissue sample collection and DNA extraction

To assess the specificity of the primers designed for this study, we needed to test their ability to amplify DNA from the target species and from all other non-target fish species that occur (or are likely to invade) upstream of the Cotter Dam. Fin clips or muscle tissue samples were collected from multiple individuals from each species (Table 2). Samples were obtained by sub-sampling fish specimens previously archived at the University of Canberra (stored frozen or in ethanol) and from specimens freshly sampled in the field (stored in 20% salt-saturated DMSO buffer). All samples were stored at -20℃ before DNA extraction.

Genomic DNA was extracted from a macerated 3-5 mm^2 fragment of the sampled tissue using a salting out extraction procedure designed for fish samples (Cawthorn *et al*., 2011). Each tissue fragment was transferred to its own 1.5 ml tube containing 400 µl tissue extraction buffer (10 mM Tris-HCL pH 8.0; 0.4

M NaCl; 2 mM EDTA), 40 µl 20% SDS and 20 µl proteinase K (10 mg/ml). Samples were then incubated overnight at 55℃ at 14 rpm. 300 µL 6M NaCl was added to each sample to precipitate proteins, before samples were centrifuged at 13,000 rpm for 30 min at room temperature. The upper aqueous phase was transferred to a new sterile tube before DNA was precipitated by adding 800 µL of 100% isopropanol and inverting for five seconds. Samples were chilled at -20℃ for 1 hour before centrifugation for 20 minutes at 13,000 rpm at 4℃. Isopropanol was aspirated and the DNA pellet was washed once with 600 µL of 70% ethanol, then centrifuged at 13,000 rpm at 4℃ for 10 min. Ethanol was aspirated and the pellet left to air dry. DNA pellets were resuspended overnight in 100 µL molecular biology grade water.

Table 2. Fish species sampled for use in primer testing.

Common Name	Species	Abbreviation used in Figures	Number of samples
Goldfish	*Carassius auratus*	C.aur	6
Two-spined blackfish	*Gadopsis bispinosus*	G.bis	7
Eastern gambusia	*Gambusia holbrooki*	G.hol	3
Trout cod	*Maccullochella macquariensis*	M.mac	4
Macquarie perch	*Macquaria australasica*	M.aus	4
Oriental weatherloach	*Misgurnus anguillicaudatus*	M.ang	2
Rainbow trout	*Oncorhynchus mykiss*	O.myk	5
Redfin perch	*Perca fluviatilis*	P.flu	2
Brown trout	*Salmo trutta*	S.tru	4

Primer testing and optimisation

The four sets of primers described above (Table 1) were tested in a series of polymerase chain reactions (PCRs) using DNA from nine fish species (Table 2). The universal fish primers were tested against DNA from all nine species, to ensure that these primers were able to successfully amplify DNA from all fish species occurring above the Cotter Dam. Each set of species-specific primers was first tested against DNA from the relevant target species, to determine the ability of the test to successfully detect that species. The species-specific primers were then tested against DNA from each of the eight non-target species, to determine the risk of non-specific amplification or false-positive results. To determine the best amplification conditions for each set of primers, PCRs were conducted across a range of annealing temperatures. To determine PCR success, 4 µl of each PCR product was visualised using agarose gel electrophoresis (gels run at 90V for 45 minutes): the presence or absence of DNA bands of the expected size (bp) indicated the success of amplification for each DNA sample under each set of PCR conditions. Negative control samples containing no DNA (water and reagents only) were included in all PCRs.

Initially, Velocity taq polymerase (Bioline) was used to test all primers. 10 µl PCRs contained final concentrations of 1x Hi-Fi buffer (Bioline), 2 mM dNTPs, 3% DMSO, 0.4 µM each of the relevant forward and reverse primers, 0.5 U Velocity taq polymerase and ~4ng/µL of template genomic DNA. PCR were conducted using an Eppendorf Mastercycler ProS thermal cycler, with cycling parameters of 98℃ for 2 min, followed by 40 cycles of 30 s at 98℃, 30 s at annealing temperature (see below) and 30 s at 72℃, followed by a final extension for 7 min at 72℃. With the aim of improving the species-specificity of these tests, the optimisation process was repeated for the *M. australasica*-specific primers using a different polymerase enzyme, MyTaq HS (Bioline). 10 µl PCRs contained final concentrations of 1x MyTaq HS Red Mix (Bioline), 0.4 µM each of the relevant forward and reverse primers and ~4ng/µL of template genomic DNA. PCR were conducted using an Eppendorf Mastercycler ProS thermal cycler, with cycling parameters of 95℃ for 2 min, followed by 35 cycles of 15 s at 95℃, 15 s at annealing temperature (see below) and 10 s at 72℃, followed by a final extension for 1 min at 72℃.

Results

Universal primers

The universal fish primers were successfully amplified from all nine fish species tested under a range of PCR conditions using the Velocity taq (Figure 1). Further testing of these primers will be required once each set of species-specific primers has been optimised, to ensure that the universal primers can be used under the same PCR conditions.

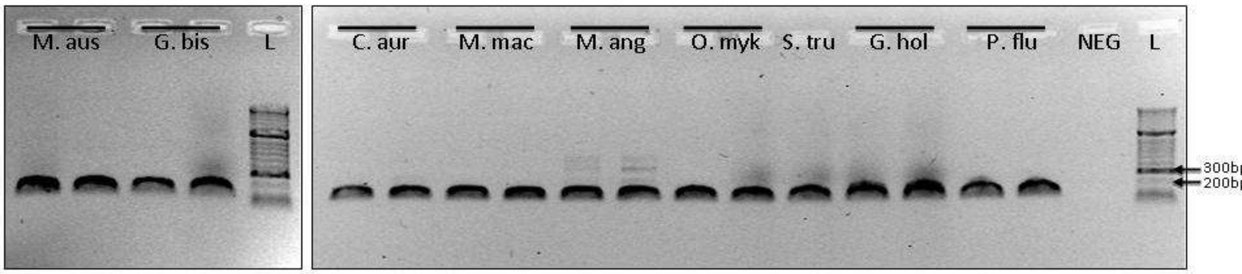

Figure 1. Gel electropherogram showing the successful amplification of DNA from nine fish species using the universal fish primers Fish_18S_1F and Fish_18S_3R (species name abbreviations explained in Table 2). A strong DNA band between the 200 bp and 300bp DNA markers is observed in PCRs from all samples except the PCR negative control. Two DNA samples were tested for all fish species except *S. trutta*, for which only one sample was available at the time of testing. *L* = DNA size standard ladder (sizes in base pairs).

G. bispinosus primers

Using the Velocity taq, we observed non-target amplification with the *G. bispinosus* species-specific primers at all annealing temperatures (Figure 2). Amplification using these primers has not yet been optimised to ensure species-specificity: additional work will be required to achieve this.

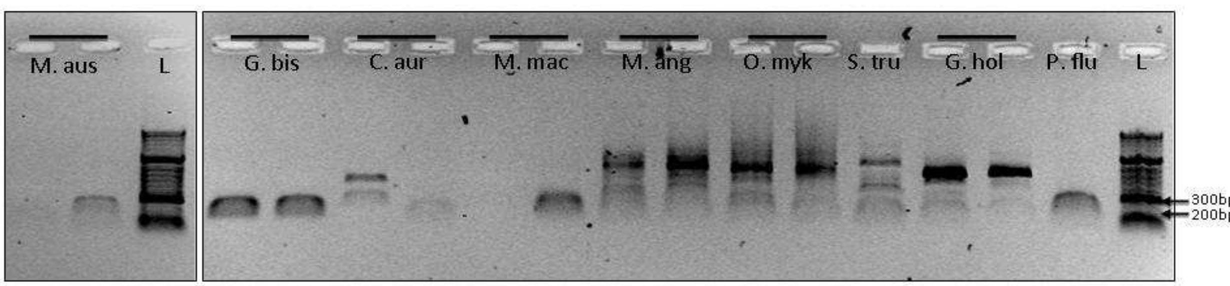

Figure 2. Gel electropherogram showing non-specific amplification of DNA from nine fish species using the *G. bispinosus* species-specific primers Gad_bis_12S_F193 and Gad_bis_12S_R310 (species name abbreviations explained in Table 2). The expected result is a strong DNA band at 118 bp in PCRs from *G. bispinosus* DNA and no DNA bands in PCRs from all other DNA samples. Instead we observed DNA bands in all species tested, in some cases multiple DNA bands from a single sample. The PCR negative controls were clean (data not shown). *L* = DNA size standard ladder (sizes in base pairs).

C. auratus primers

Using the Velocity taq, we observed non-target amplification with the *C. auratus* species-specific primers at all annealing temperatures (Figure 3). Amplification using these primers has not yet been optimised to ensure species-specificity: additional work will be required to achieve this.

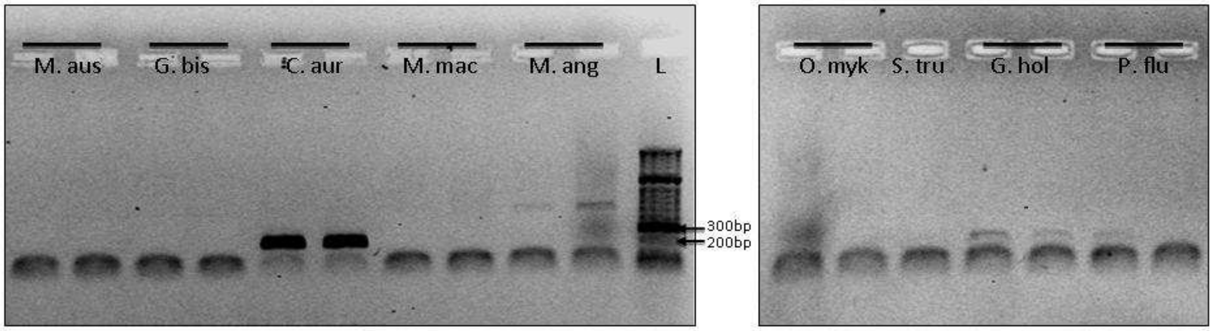

Figure 3. Gel electropherogram showing non-specific amplification of DNA from nine fish species using the *C. auratus* species-specific primers Car_aur_12S_F9 and Car_aur_12S_R227 (species name abbreviations explained in Table 2). The expected result is a strong DNA band at 219 bp in PCRs from *C. auratus* DNA and no DNA bands in PCRs from all other DNA samples. We observed strong, clear DNA bands at the expected size in PCRs from *C. auratus* DNA, but we also observed fainter DNA bands in PCRs from several other species, including *M. anguillicaudatus*, *O. mykiss*, *G. holbrooki* and *P. fluviatilis*. The PCR negative controls were clean (data not shown). *L* = DNA size standard ladder (sizes in base pairs).

M. australasica primers

Using the Velocity taq, we observed non-target amplification with the *M. australasica* species-specific primers at all annealing temperatures. Consequently we tested these primers with a different enzyme, MyTaq HS, with the aim of improving the species specificity of these primers. Using the MyTaq HS enzyme, the *M. australasica* primers reliably amplified a product of the expected size (205 bp) up to an annealing temperature of 64.5℃. Some amplification was also observed with an annealing temperature of 65.5℃, but this was too weak to serve as a reli able species identification test (Figure 4). Some non-specific amplification of DNA from other fish species, including *G. holbrooki* and *P. fluviatilis* was detected at lower annealing temperatures between 55℃ and 62℃. However, at higher annealing temperatures above 63.5℃, only DNA from *M. australasica* was amplified (Figure 5).

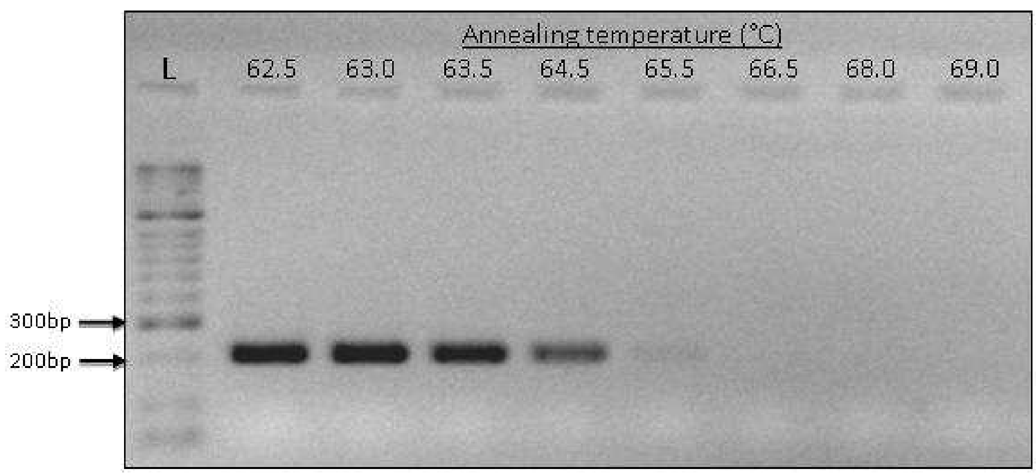

Figure 4. Gel electropherogram showing amplification of DNA from a single *M. australasica* DNA sample using the *M. australasica* species-specific primers Mac_aus_12S_F22 and Mac_aus_12S_R226 under a range of different PCR annealing temperatures. The expected result is a strong DNA band at 205 bp. We observed strong, clear DNA bands at the expected size in PCRs with annealing temperatures up to 64.5℃. *L* = DNA size standard ladder (sizes in base pairs).

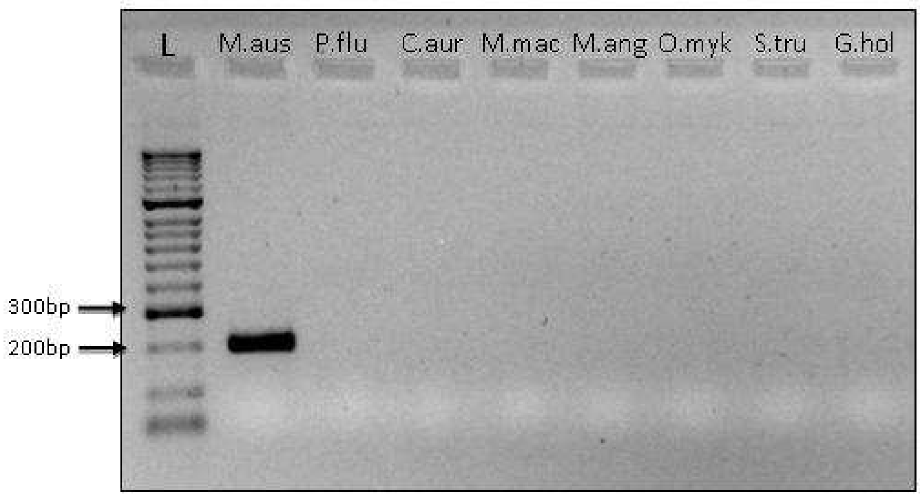

Figure 5. Gel electropherogram showing amplification of DNA from eight fish species (species name abbreviations explained in Table 2) using the *M. australasica* species-specific primers Mac_aus_12S_F22 and Mac_aus_12S_R226 with a PCR annealing temperature of 63.5℃. The expected result is a strong DNA band at 205 bp in PCRs from *M. australasica* DNA and no DNA bands in PCRs from all other DNA samples. *L* = DNA size standard ladder (sizes in base pairs).

Discussion

DNA detection holds great potential for addressing ecological questions that are difficult to answer using morphological data alone. In this case, we aim to use DNA detection methods to investigate predation of native freshwater fish species by introduced trout. The availability of reliable DNA tests will make a valuable contribution to the conservation management of these native fish by determining the level of threat posed to these species by alien predators.

We have designed new DNA tests for two species of threatened native fish and have demonstrated the species specificity of one of these tests, for Macquarie perch. We are confident that additional investment of effort in the second DNA test, for two-spined blackfish, would enable us to optimise this test to improve its species specificity. This work would require additional PCR tests under a range of PCR conditions and temperatures using the MyTaq HS enzyme.

We note that the PCR tests we have developed may not be species-specific outside the target area of the Cotter Reservoir and River upstream of the Cotter Dam. This is because DNA sequence data were not available for all Australian freshwater fish species at the time of primer design and sequences were only widely available for a small number of genes. Thus primer design was limited by the availability of the sequence data. In some cases, non-target fish species that occur elsewhere in the Murray-Darling Basin have DNA sequences very similar to those used here for species-specific primer design. For example the *M. australasica* 12S rRNA gene sequence is almost identical to that of its close relative golden perch *M. ambigua*. If the *M. australasica* PCR test were used in an area where *M. ambigua* may also occur, it is likely that *M. ambigua* DNA would also give a positive result using this test. In these circumstances we would recommend extensive testing of the species-specific primers against a wider range of fish species, including all species present in the study area, to identify any potential sources of false-positive results.

Now that a PCR test is available to enable detection of Macquarie perch, further research will be required to understand the effectiveness of and the limitations to this test when analysing unknown samples. These measures will also be required for the two-spined blackfish test once that has been successfully optimised. Additional funding will be required to enable completion of this work and the application of these DNA tests to unknown samples taken from the Cotter Reservoir and River. The issues that should be addressed in future research include:

- account for the possibility of intra-specific genetic variation at the PCR primer sites, which could reduce the effectiveness of this test. This could be achieved by testing DNA from a greater number of *M. australasica* individuals from a wider range of geographic locations.

- determine the most appropriate DNA extraction methods to apply to trout stomach contents to enable the detection of prey DNA. This could be achieved by testing a range of DNA extraction methods followed by PCR tests using the universal mammal primers and the *C. auratus*-specific primers (once these have been fully optimised) to detect prey DNA from stomach contents of trout known to have fed on goldfish.

- assess the sensitivity of the PCR test when applied to trout stomach contents by analysing DNA from known samples. These should include multiple negative controls (DNA from stomach contents of trout that have never encountered *M. australasica*) and positive controls (DNA from stomach contents of trout that have been fed known quantities of *M. australasica* larvae under a known timeframe). Variables to be considered for successful DNA detection of *M. australasica* include the quantity of *M. australasica* DNA ingested and the minimum level of detection possible (i.e. need to assess PCR success relative to the number of *M. australasica* larvae consumed) and the time taken for *M. australasica* DNA to pass through the digestive tract of the trout (i.e. need to determine for how long after feeding the *M. australasica* DNA is detectable).

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