Check for updates

Effects of habitat and pulp and paper mill contamination on a population of brook stickleback (*Culaea inconstans*)

Gillian Z. MacDonald, Natacha S. Hogan and Michael R. van den Heuvel

ABSTRACT

This study examined the responses of a population of brook stickleback (*Culaea inconstans*) exposed to pulp mill effluent at Jackfish Bay, Lake Superior, Canada, in May 2007 and May 2011. Brook stickleback were extirpated from the effluent-receiving site, presumably due to anoxia after this period. Females at the effluent-receiving site had significantly larger gonad sizes in 2007 and 2011. In 2011, effluent-exposed female gonadal development was significantly advanced when compared with reference sites; they were the second most mature when compared among three different reference sites. Analysis of 7-ethoxyresorufin-*O*-deethylase activity revealed that effluent-receiving site females had greater CYP1A induction in 2007 and significantly greater CYP1A induction in 2011. Effluent-receiving site males showed significantly reduced CYP1A induction in 2007 and significantly greater induction in 2011. Chemical evaluation of sediment from the receiving environment showed elevated levels of resin acids and the polycyclic aromatic hydrocarbon, retene. Higher condition factors and more mature gonads were consistent with higher winter and spring temperatures modified by effluent or by lake vs. stream environments. Overall, effects on effluent-exposed brook stickleback were not consistent with reported effects in white sucker exposed to the same effluent in previous studies.

Key words | fishes, oxygen, pulp and paper, reproduction, temperature, polycyclic aromatic hydrocarbon

Gillian Z. MacDonald
Michael R. van den Heuvel (corresponding author)
Canadian Rivers Institute, Department of Biology,
University of Prince Edward Island,
Charlottetown,
Canada
E-mail: *mheuvel@upei.ca*

Natacha S. Hogan
Toxicology Centre,
University of Saskatchewan,
Saskatoon,
Canada

This article has been made Open Access thanks to the kind support of CAWQ/ACQE (https://www.cawq.ca).

INTRODUCTION

Downstream of pulp and paper effluent discharge, deleterious effects on fish populations such as reductions in sex steroid hormone levels, gonad size and fecundity, changes in secondary sex characteristics, and delayed maturity have been observed (Munkittrick *et al.* 1992; Sandström & Neuman 2003; McMaster *et al.* 2006; van den Heuvel 2010). A review of two decades of research on reproductive effects of pulp mill effluents on fishes attributes such effects to all types of mill processes, such as bleached kraft, bleached sulphite, mechanical, multiprocess, and thermo-mechanical (Hewitt *et al.* 2008).

Despite decades of research, and the accumulation of considerable knowledge on the mechanism of reproductive effect, understanding of the causative agents of those effects is not conclusive. It is known that compounds with androgenic and oestrogenic activity, likely derived from wood, have been detected in pulp mill effluents (van den Heuvel *et al.* 2010). Reproductive impacts in fish may also be attributed to neuroendocrine disruption (Basu *et al.* 2009) or occur indirectly via nutritional deficiencies (van den Heuvel *et al.* 2010). Several recent studies have also consistently demonstrated an association between effluent biochemical oxygen demand (BOD) and decreased fathead minnow (*Pimephales promelas*) spawning success (Martel *et al.* 2011; Kovacs *et al.* 2011, 2013).

This is an Open Access article distributed under the terms of the Creative Commons Attribution Licence (CC BY 4.0), which permits copying, adaptation and redistribution, provided the original work is properly cited (http://creativecommons.org/licenses/by/4.0/).

doi: 10.2166/wqrj.2019.005

Downloaded from http://iwaponline.com/wqrj/article-pdf/55/1/52/709313/wqrjc0550052.pdf
by guest

In 1987, Jackfish Bay, Lake Superior was deemed an area of concern under the Canada–United States Great Lakes Water Quality Agreement due to the effluent it received from a bleached kraft pulp mill in nearby Terrace Bay, Canada (JBRAP 1998). Monitoring efforts over two decades (1988–2007) revealed that effluent-exposed wild white sucker (*Catostomus commersoni*) consistently have smaller gonad sizes, reduced circulating sex steroid levels, increased liver size and condition factor (CF), increased age, delayed maturity, and increased CYP1A induction, when compared with unexposed wild white sucker (Munkittrick *et al.* 1991, 1992, 1994; Bowron *et al.* 2009). This pattern of metabolic disruption in Jackfish Bay is consistent with a national pattern of response from over 200 fish surveys conducted in three cycles of the Canadian Environmental Effects Monitoring (EEM) program between 1992 and 2004 (Barrett *et al.* 2010). As the Terrace Bay mill added secondary treatment facilities and converted to elemental chlorine-free (ECF) bleaching, some of the observed effects in white sucker became less pronounced (Bowron *et al.* 2009). For example, gonad size differences and liver size differences between wild-exposed and wild-reference white sucker were reduced although differences in the condition remained consistent. CYP1A induction, although reduced, remained significantly higher in wild-exposed than in reference white sucker.

Despite the extensive studies on effluent impacts in the Lake Superior-receiving environment, no examination of fish populations has been conducted in the receiving environment upstream of where effluent enters Lake Superior. This environment includes 14 km of stream, the origin of which is the effluent discharge, including a number of small lakes. The settlement of solids in this region has likely contributed to high levels of legacy contaminants such as chlorinated dioxins (Sherman *et al.* 1990). In such a depositional environment, the microbial production of the polycyclic aromatic hydrocarbon (PAH) retene – a known inducer of CYP1A enzymes in fish (Fragoso *et al.* 1998; Billiard *et al.* 1999; Brinkworth *et al.* 2003) – via anaerobic degradation in lake sediments is expected (Rämänen *et al.* 2010).

The main objective of this study was to examine for physiological and biochemical responses in a small forage fish, the brook stickleback (*Culaea inconstans*), exposed to effluent from the Terrace Bay pulp and paper mill. It was hypothesized that in small depositional lakes receiving high concentrations of pulp mill effluent, sediment contamination might result, including the production of the PAH retene. It was further hypothesized that given much higher effluent load when compared with the Lake Superior-receiving environment, biochemical, physiological and reproductive impacts may be detectable in this population of brook stickleback. These hypotheses were examined by measuring sediment chemistry and a number of standard environmental variables in three reference locations and in the receiving environment upstream of Lake Superior. Brook stickleback population, physiological, and biochemical parameters were measured in the receiving environment and the three reference locations between 2007 and 2013.

METHODS

Brook stickleback capture locations

Moberly Lake contained a population of brook stickleback at the time the study was initiated, and received effluent from the bleached kraft mill located in Terrace Bay, Ontario, Canada (Figure 1). During mill operational periods, effluent flow in 2012 averaged 85,000 m^3 per day, reduced from about 102,000 m^3 per day in 2008 and, during operational years between 2007 and 2014, pulp output averaged 326,000 air-dried metric tonnes per annum 2008 (Traci Bryar, Environmental Superintendent at AV Terrace Bay, personal communication). The Terrace Bay Mill uses ECF bleaching and effluent treatment consists of a primary treatment system that removes fibres and suspended solids and a secondary treatment system of an aerated lagoon (Munkittrick *et al.* 1992). The aerated lagoon has an 8–10-day retention time and upon its implementation, it significantly reduced BOD (by 95%), TSS (by 29%), and AOX (by 29%) (Munkittrick *et al.* 1992).

The mill discharges its effluent into Lake Superior via Blackbird Creek (13.7 km). Prior to release into Lake Superior, the effluent passes through a number of small lakes – the largest and final lake in the system is Moberly Lake, about 1.9 km in length. In the early 1940s, Lake A was 6.1 m at its deepest point and covered an area of about 19 ha (JBRAP 1998). Over time, the deposition of wood fibres from the mill caused the lake to be partially

Downloaded from http://iwaponline.com/wqrj/article-pdf/55/1/52/709313/wqrjc0550052.pdf
by guest

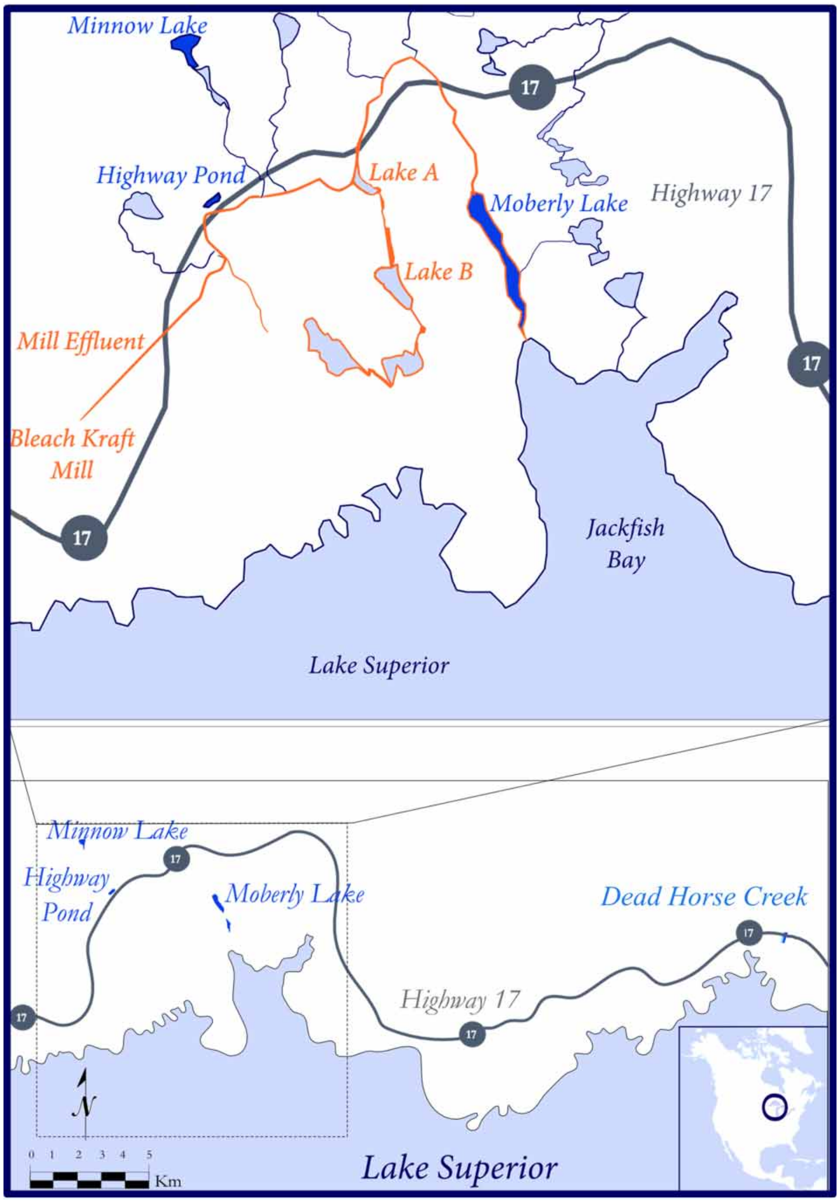

Figure 1 | Map of study area. Mill effluent flows from kraft mill to Moberly Lake and into Lake Superior.

filled in, redirecting Blackbird Creek around it and into Moberly Lake in the early 1980s (JBRAP 1998). Prior to redirecting Blackbird Creek, Moberly Lake was approximately 28 ha and 6.4 m at its deepest point (JBRAP 1998). Since 1994, maximum depth has decreased to 5 m and the area covered has also been reduced (JBRAP 1998). Since 2006, the mill has experienced three long-term shutdowns: for 7 months in 2006, for 19 months in 2009–2010, and again for 10 months in 2011–2012 (Figure 2).

Three sites were chosen in addition to Moberly Lake and used as reference sites for this study. The smallest site is an unnamed pond in a small tributary of Blackbird Creek not receiving the effluent and isolated from the main branch of Blackbird Creek by beaver impoundment; this site will henceforth be referred to as Highway Pond. Highway Pond was used as a reference site in spring 2011. Minnow Lake (0.09 km^2) is a shallow lake, similar to Moberly Lake (0.27 km^2), and is also on the Blackbird

Downloaded from http://iwaponline.com/wqrj/article-pdf/55/1/52/709313/wqrjc0550052.pdf
by guest

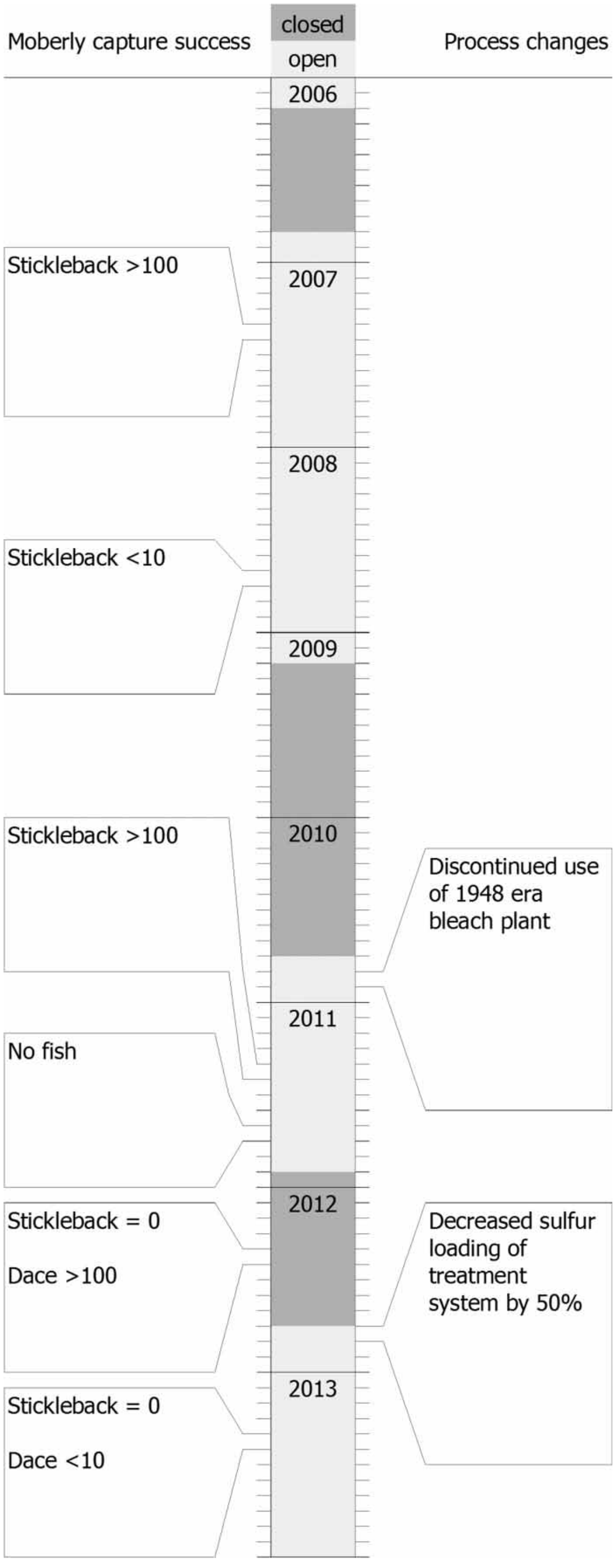

Figure 2 | Outline of operational history at the Terrace Bay pulp and paper mill and brook stickleback sampling periods.

Creek system about 3 km upstream of the main branch of the creek. The brook stickleback population in Minnow Lake was isolated from the Blackbird Creek water through a series of beaver impoundments and unfavourably fast water. Minnow Lake was used as a reference site in spring 2011. Dead Horse Creek is a more stream-like environment with a beaver impoundment containing brook stickleback in a separate watershed approximately 20 km away from the other sites. It was used as a reference site in spring 2007 and 2011.

Water quality

In May 2011, TidbiT v2 UTBI-001 temperature loggers (Onset, Cape Cod, MA, USA) were deployed at 1–1.5 m water depth (at the time of snowmelt) in Moberly Lake, Dead Horse Creek, and Minnow Lake (the population at Highway Pond was only discovered at this time and a temperature logger was not available to deploy). Loggers were programmed to record the temperature every 4 h. Loggers were cased in an ABS plastic plumbing tee fitted with two permanent adapters with openings smaller than the TidbiT unit to allow for water flow. This entire system was attached to a concrete block with aircraft cable, and the block was attached to a solid tree on shore via a chain.

Temperature, dissolved oxygen (DO), conductivity, and pH were recorded using a handheld YSI professional plus multiparameter water quality meter equipped with a model 10102030 quad sensor cable sonde (YSI Inc., Yellow Springs, OH, USA) during all sampling trips (May 2011, September 2011, May 2012, and May 2013). Using conductivity measurements taken from Blackbird Creek and the pulp mill effluent conductivity, the concentration of effluent along Blackbird Creek was estimated from the mill to Moberly Lake. The concentration of effluent was determined using conductivity measurements as shown in Equation (1), where Λ is conductivity in μS/cm. An estimate of the conductivity of diluent water was determined by averaging the conductivity of four tributaries just prior to their inflow into the Blackbird Creek system. Blackbird Creek originates as the mill effluent release, so there was no 'upstream' diluent water to sample and the tributaries measured to represent the average conductivity of most of the diluent water into Blackbird Creek. The mean conductivity of diluent water

Downloaded from http://iwaponline.com/wqrj/article-pdf/55/1/52/709313/wqrjc0550052.pdf
by guest

was 144 μS/m, with a range of 60–300 μS/m

$$\frac{\Lambda_{\text{MoberlyLake}} - \Lambda_{\text{Average source water}}}{\Lambda_{\text{Effluent}} - \Lambda_{\text{Average source water}}} \times 100 \tag{1}$$

Sediment chemistry

Sediment cores for chemical analysis were collected in 2007 with a 10 cm plastic tube. Five cores were collected in the south part of Moberly Lake adjacent to the brook stickleback capture area. As the 2007 analysis was meant only as an exploratory evaluation of the concentration of sediment extractives at a known pulp mill-contaminated site, samples were collected from reference locations. Sediment cores were again collected in Moberly Lake in 2010 and Minnow Lake was added in order to examine levels of retene, the major extractive in the region, in an environment not receiving pulp and paper effluent. For both sampling periods, a sediment pool for analysis was made from the five cores using the top 10 cm of each core.

For the 2007 analysis, the freeze-dried sediment was mixed with granular sodium sulphate (BDH, UK) at a 1:1 ratio (w:w) and then ground with a mortar and pestle. The mixture was spiked with 10 μL of recovery standard containing d10-anthracene as a neutrals surrogate, dihydrocholesterol as a sterol surrogate and 8(14)-abietenic acid as a resin acid surrogate. The sediment was extracted using an Agilent 7680T supercritical fluid extractor. Three successive extraction steps of 25 min using CO_2 at 227 bar and 70 °C and a flow rate of 3.0 mL/min were employed to extract the analytes. After each extraction step, the analytes were trapped on a C18 column and then eluted using dichloromethane that was dried over sodium sulphate. The residue was transferred to 250 μL glass inserts housed in 1.8 mL screw-cap autosampler vials (Alltech, New Zealand). After the addition of dibromoanthracene (TCI, Japan) in pyridine as an internal standard, the samples were derivatized by adding 50 μL of bis(trimethylsilyl)trifluoroacetamide (BSTFA) +1% trimethylchlorosilane silylation reagent (Alltech, New Zealand) and heating for 1 h at 70 °C.

Analysis of the extracts was performed on a 6890N gas chromatograph (GC) and 5973N electron impact ionization mass selective detector (Agilent Technologies, USA) acquiring data in the full scan (50–800 *m/z*) mode. An autosampler was used to introduce 1 μL of the sample into a purged splitless injector maintained at 280 °C. The analytes were separated on an Agilent Ultra-2 column (50 m × 0.2 mm ID, film thickness 0.33 μm) with helium as a carrier gas. The oven parameters used were 60 °C (1 min), 10 °C/min until 205 °C then 3 °C until 270 °C and 6 °C/min until 300 °C (45 min). Final concentrations were corrected for the recovery of surrogate standards.

In 2010, the detailed GC–MS analysis was no longer available to investigators and only the substance retene was analysed to provide a comparison. The sediment was dried by mixing with sodium sulphate and extracted for 24 h using Soxhlet extraction with dichloromethane. Samples were evaporated and made up to 1 mL in methanol. Retene was analysed by HPLC using a C-18 column with fluorescence detection (excitation 260 nm, emission 380 nm) and quantified against a retene standard (Sigma). Retene recovery using this method was found to be 95–98%.

Fish capture

Minnow traps (40 cm long, 20 cm in diameter, made of 0.5 cm galvanized mesh with 2.5 cm openings) were used as the primary method of capture. Minnow traps were baited with cat food that was free for fish to feed upon and set in the habitat that appeared desirable for nesting males (quantities of leaves and debris). Occasionally, electrofishing using a Smith-Root LR-24 Backpack Electrofisher was used to supplement minnow trapping in order to attain the required number of brook stickleback. Traps were left overnight and checked by 1 pm of the following day. In the spring seasons, capture occurred during the first week of May which was chosen as this is the earliest that snow and ice conditions would allow fish capture. In fall 2008 and 2011, the capture was attempted during the last week of September which was chosen as the end of the growth period to obtain a measure of gonadal development and prior to the period where ice would prevent fish capture.

Fish sampling

Fish were transferred into 20 L buckets containing aerated water from the site of capture and transported a short distance to a sampling area. Fish were euthanized with a

Downloaded from http://iwaponline.com/wqrj/article-pdf/55/1/52/709313/wqrjc0550052.pdf
by guest

sharp blow to the head followed by spinal severance. Weight and fork length were recorded to the nearest tenth of g and mm, respectively. Livers were excised, weighed, and divided into two parts; approximately 20 mg of tissue was stored in 200 μL of RNA later and the remainder was flash frozen in liquid nitrogen. Gonads were also excised and weighed. Whole gonads were placed in histocassettes, fixed in 10% neutral-buffered formalin, and later transferred to 70% ethanol. Carcasses were placed in individual Whirl-Pak® bags, flash frozen, and stored at −20 °C.

7-Ethoxyresorufin-*O*-deethylase analysis

7-Ethoxyresorufin-*O*-deethylase (EROD) analysis was conducted using a modification of the fluorescence plate reader technique outlined by van den Heuvel *et al.* (1995) as a catalytic measure of CYP1A. The entire fish liver (~50 mg) was homogenized in 500 μL of cryopreservative buffer (0.1 M phosphate, 1 mM EDTA, 1 mM dithiothreitol, and 20% glycerol, pH 7.4) using a sonic dismembrator (Branson SLPe, Connecticut, USA). The homogenized solution was then centrifuged at 9,000 × *g* to obtain the post mitochondrial supernatant (PMS) containing cellular proteins. The EROD reaction contained 0.1 M HEPES buffer (pH 7.8; Sigma), 5.0 mM Mg^{2+}, 0.5 mM NADPH (Sigma), 1.5 M 7-ethoxyresorufin (Sigma), and 0.5 mg/mL of PMS protein. Reactions were allowed to occur for 10 min and then terminated with acetonitrile and read on a fluorescence plate reader (Bio-Tek FLx800; 530 nm excitation, 590 nm emission). Protein content was estimated from fluorescamine fluorescence (390 nm excitation, 460 nm emission filters) against a bovine serum albumin standard (Sigma) and activity corrected for protein concentrations.

Histological analysis

Gonads were embedded in paraffin, sectioned along the coronal plane to about 4–6 μm thick, placed on slides, stained with haematoxylin and eosin, and then permanently mounted for viewing under a compound microscope. The stage of maturity was determined based on methods for fathead minnows published by USEPA (2006). All samples were coded to ensure blind analysis of histological data. All oocytes in the ovarian section were counted. Individual oocytes were categorized as being perinuclear, cortical alveolar, early vitellogenic, late vitellogenic, or mature.

Statistics

All data for parametric analysis were evaluated for normality using normal probability plots. Weight, liver size, and gonad size were analysed using analysis of covariance (ANCOVA), with logarithmically transformed values to meet the assumptions for normality, using length (weight) or body weight (liver, gonad), plus the categorical treatment variables. Somatic data were expressed as indices for presentation purposes using the least square means and covariate means from the ANCOVA. Gonadosomatic index is defined as the percent of body weight that is made up of gonads, and liver somatic index is defined as the percent of body weight that is made up of liver. Condition factor is a ratio of fish body weight to length ($k = 100 \times \text{weight/length}^3$). ANOVAs were performed to detect differences among sites in EROD induction. Tukey's *post hoc* tests were conducted to make pairwise comparisons among sites when more than two sites were being compared. All ANOVAs and ANCOVAs were performed on SYSTAT version 13.0 (Systat Software, San Jose, CA, USA). To evaluate the overall pattern of the relative frequency of ovarian stages between sites, an ANOSIM based on the Bray–Curtis similarity of the relative proportions of each ovarian stage was performed using 999 permutations using PRIMER, v6 software (2006 PRIMER-E Ltd, Plymouth, UK). The critical level of statistical differences for all analyses was $\alpha = 0.05$.

RESULTS

Water quality

At Moberly Lake, DO was 60–95% saturated during the first week of May in 2011, 2012, and 2013 (Table 1). During the mill operational period in the last week of September 2011, DO decreased to 7%. DO was relatively consistent among references sites within seasons, usually falling between 70 and 115%, this is excepting Dead Horse Creek's super-saturation to 153% in May 2011 and Minnow Lake's low of 18.3% in May 2011 (Table 1). Conductivity in Moberly

Downloaded from http://iwaponline.com/wqrj/article-pdf/55/1/52/709313/wqrjc0550052.pdf
by guest

Table 1 | Water quality measurements during stickleback sampling periods

Date	Site	Temperature (°C)	DO (%)	DO (mg/L)	Conductivity (µs/cm)	pH	Effluent dilution (% v/v)
May 2011	Moberly	9.4	61.2	7.0	474	7.66	28
	Dead Horse Creek	1.8	153.3	21.3	32	7.51	
	Highway Pond	4.1	87.2	11.3	160	7.53	
	Minnow Lake	3.4	18.3	2.4	35	7.50	
September 2011	Moberly	15.2	7.2	0.7	1,024	7.51	72
	Dead Horse Creek	12.1	114.1	12.2	75	7.19	
	Highway Pond	13.7	73.0	7.6	571	6.74	
	Minnow Lake	15.1	110.5	11.1	727	7.55	
May 2012	Moberly Lake	7.2	95.8	11.7	152	7.72	57
	Dead Horse Creek	13.1	97.4	10.2	50	8.13	
	Highway Pond	14.9	98.2	10.0	498	7.66	
	Minnow Lake	11.2	NA	NA	NA	NA	
May 2013	Moberly Lake	3.9	93.5	12.2	527	7.89	25
	Dead Horse Creek	0.1	103.1	15.0	31	7.62	
	Highway Pond	0.1	86.7	12.6	378	8.25	
	Minnow Lake	3.4	81.1	10.8	35	5.75	

NA, data not collected during this period.

Lake was consistently higher (1.4–17 times) than reference sites during mill operational periods. Upon the mill becoming non-operational in December 2012 (Figure 2), Moberly Lake's conductivity fell to 152 µS/cm, similar to the mean diluent steam conductivity of 144 µS/cm (Table 1). Based on calculations, effluent concentrations in Moberly Lake were 28, 72, 57, and 25% in May 2011, September 2011, May 2012, and May 2013, respectively (Table 1).

At Moberly Lake, the water temperature was affected by whether or not the mill was operational. Generally, while the mill was operational, the temperature increased 3–5 °C above the temperature of reference sites. For example, during the non-operational period (2 December 2011 until 11 October 2012; Figure 3), Moberly Lake temperature was very similar to Minnow Lake during the summer months, although somewhat warmer than the more lotic Dead Horse Creek at this time. During the winter period of the shutdown from December 2011 to April 2012, Moberly Lake's temperature profile aligned more closely with that of Dead Horse Creek. During that winter period, Minnow Lake exhibited warmer ~4 °C temperature typically found under ice (none of the systems studied freeze entirely). The presence of effluent during the operational period resulted in Moberly Lake temperatures being warmer in the winter of 2012/2013 compared with 2011/2012 by about 3 °C.

Sediment analysis

In 2007, Moberly Lake sediment samples contained 2,375.8 µg/g dry weight of resin acid neutrals, 270.5 µg/g dry weight of resin acids, and 406.8 µg/g dry weight of phytosterols (Table 2). The neutrals were the dominant extractives in this sediment, and the PAH retene comprised a majority of this at 1,625.1 µg/g dry weight. Abietic acid was the most dominant resin acid and sitosterol was the most dominant sterol. The neutrals are derived from the bacterial decomposition of resin acids and the sum total of the neutrals represents 91% of the total of both neutrals and resin acids, representing a substantial conversion of resin acids to neutrals. In 2010, the measured retene concentration in the Moberly Lake sediment was 833.0 µg/g. As a reference, Minnow Lake sediments contained 4.3 µg/g dry weight of retene.

Presence/absence of fish

Brook stickleback were generally more difficult to capture during the September capture efforts in 2008 and 2011 when compared with spring sampling periods in 2007 and 2011. While fish were still present at all three reference sites in September 2011, exhaustive minnow trapping and seine netting efforts at Moberly Lake in September 2011,

Downloaded from http://iwaponline.com/wqrj/article-pdf/55/1/52/709313/wqrjc0550052.pdf
by guest

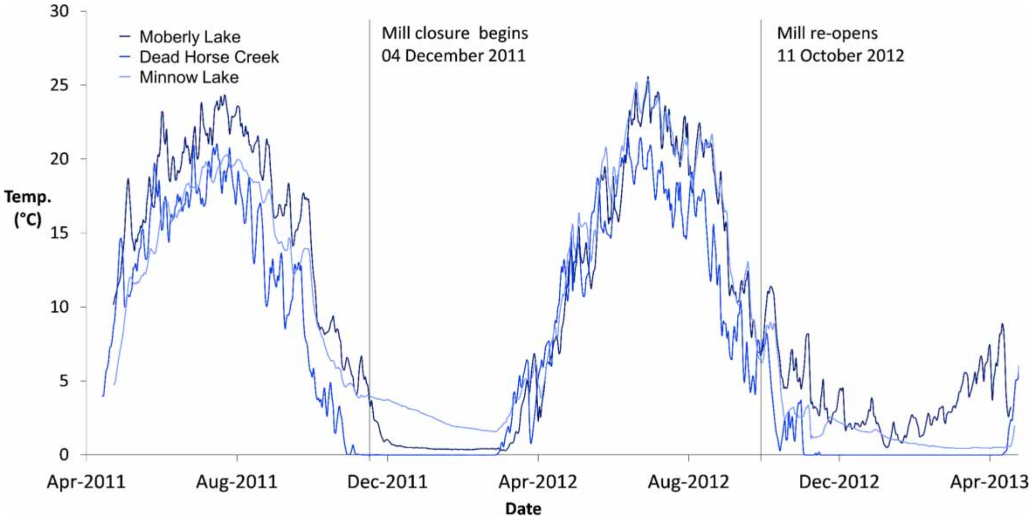

Figure 3 | Daily average temperature at 1–1.5 m depth at the sampling sites measured every 3 h.

May 2012, and May 2013 indicated that brook stickleback were no longer present in the effluent-exposed site. Sampling effort was carried out over 3 days with more than 90 overnight minnow trap sets along the full 2 km of Moberly Lake at each sampling period. In addition, at least 20 seine net hauls were made without a single stickleback captured. This absence corresponded with the low DO levels measured during September 2011 (7% saturation) and considering the temperature in September had already dropped 10 °C from the high of nearly 25 °C experienced that summer, extended periods of anoxia were likely. In May 2012, brook stickleback remained highly abundant in Minnow Lake. While stickleback were no longer present in Moberly Lake and unidentified species of cyprinid, thought to be a species of dace, was captured in May of 2012 and 2013, but not in September 2011.

EROD analysis

EROD analysis showed inconsistent results between the two sampling years (Figure 4). Moberly Lake male brook stickleback had 1.9-fold higher EROD induction than the pooled reference fish in 2011; however, in 2007, Dead Horse Creek male brook stickleback had 2.5-fold higher EROD induction compared with fish from Moberly Lake. The trend in females was more consistent; Moberly Lake females had higher EROD induction in both sampling years (2.3-fold higher in 2011 and 1.4-fold higher in 2007). However, this elevation in females was only significantly different in 2011.

Somatic parameters

Gonad size as it covaries with fish weight in females from Moberly Lake, the effluent-receiving site, was significantly higher compared with fish from the reference sites in 2007 and 2011, with the exception of Minnow Lake in May 2011 (Figure 5(a)). Minnow Lake brook stickleback gonad size was significantly higher than Moberly Lake females in 2011. There were no significant differences in gonad size in Moberly Lake male brook stickleback when compared with any of the other populations during 2007 or 2011. However, liver size as it covaries with body weight in male brook stickleback was consistently higher in fish from Moberly Lake, the effluent-receiving area, in both May 2007 and May 2011 (Figure 5(d)). The same pattern was observed for female fish (Figure 5(c)), except when compared with Minnow Lake in May 2011 where there was no significant difference in liver size between the Minnow Lake and Moberly Lake populations. While females from Moberly Lake consistently had the highest body weight as it covaries with body length, this difference was only statistically significant in 2011 (Figure 5(e)). Males did not show any consistent differences for body condition (Figure 5(f)). In both males and females, the average fork length of both male (Figure 5(h)) and female (Figure 5(g)) brook

Downloaded from http://iwaponline.com/wqrj/article-pdf/55/1/52/709313/wqrjc0550052.pdf
by guest

Table 2 | Sediment chemistry profile of Moberly Lake, May 2007 including resin acid neutrals, resin acids, and phytosterols

Compound	Concentration (μg/g dw)
Resin acid neutrals	
Fichtelite	703.2
Dehydroabietin	17.6
Tetrahydroretene	8.4
Retene	1,625.1
Methyldehydroabietin	21.5
Total resin acid neutrals	2,375.8
Resin acids	
Pimaric acid	4.0
Sandaracopimaric acid	1.1
Isopimaric acid	4.0
Palustric acid	0.5
Dehydroabietic acid	31.0
Abietic acid	56.6
Neoabietic acid	0.1
Pimarenic acid	5.3
Sandaracopimarenic acid	20.0
Isopimarenic acid	n.d.
13-Abietenic acid	2.8
Pimaranic acid	11.8
Isopimaranic acid	1.6
Abietanic acid	79.0
Seco-1-dehydroabietic acid	1.5
Seco-2-dehydroabietic acid	0.8
12-Chlorodehydroabietic acid	12.5
14-Chlorodehydroabietic acid	7.1
12,14-Dichlorodehydroabietic	28.8
7-Oxodehydroabietic acid	2.2
Total resin acids	270.5
Phytosterols	
Cholesterol	20.9
Campesterol	22.8
Stigmasterol	28.4
Sitosterol	243.7
Sitostanol	91.0
Total phytosterols	406.8
Total extractives	3,461.0

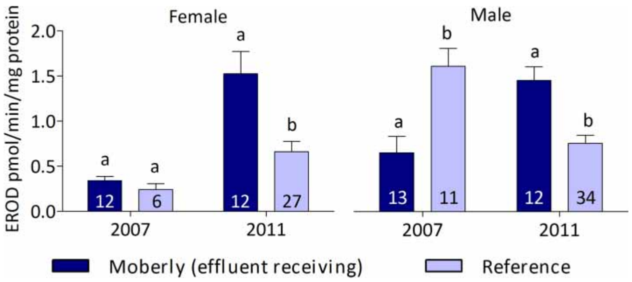

Figure 4 | CYP1A induction in wild brook stickleback as measured by EROD analysis. In 2007, the only reference site is Dead Horse Creek. In 2011, the reference sites – Dead Horse Creek, Highway Pond, and Minnow Lake – were pooled as there were no significant differences among sites. Significance indicators are for between sites within seasons.

stickleback in Moberly Lake was significantly greater than the fork length of brook stickleback in Dead Horse Creek in 2007. No significant differences in fork length were seen among any fish samples from 2011, except that Minnow Lake males were significantly shorter than males from all other sites in 2011. Stickleback typically only lives for 2 years; thus, fork length of these individuals represents their maximum growth.

Gonadal development

An ANOSIM on the relative frequency of ovarian development stages of female fish showed that all sites were significantly different from one another with the exception of Dead Horse Creek and Highway Pond (Figure 6). Of the four populations, ovaries from Minnow Lake females were the most developed and different from the other site with global *R*-values (1 being no similarity between groups and 0 indicating that groups are identical) of 0.852, 0.761, and 0.404 when compared with the Dead Horse Creek, Highway Pond, and Moberly Lake populations, respectively. Ovaries from the Moberly Lake females also had significantly advanced maturation when compared with Dead Horse Creek and Highway Pond with global *R*-values of 0.456 and 0.169 for these comparisons, respectively. Only Dead Horse Creek and Highway Pond, the least developed of the sites, were not significantly different, with a global *R*-value of 0.062.

DISCUSSION

The discharge of the bleach kraft pulp mill in Terrace Bay resulted in high levels of the PAH retene in a depositional zone although brook stickleback liver CYP1A levels did

Downloaded from http://iwaponline.com/wqrj/article-pdf/55/1/52/709313/wqrjc0550052.pdf
by guest

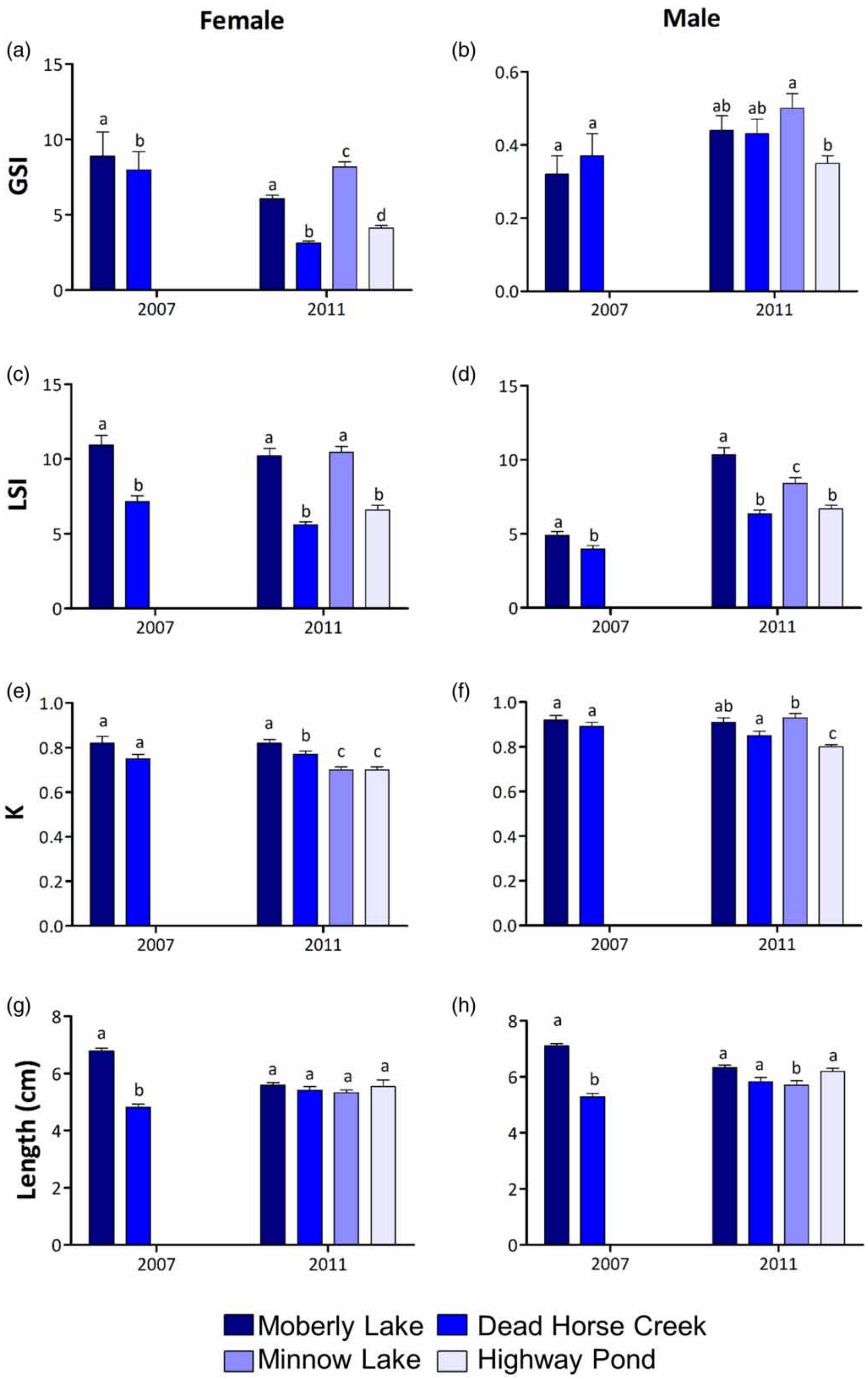

Figure 5 | Somatic measurements of brook stickleback sampled in May 2007 and May 2011. Data are expressed as mean (+SEM). Graphs (a) and (b) show the gonad somatic index, (c) and (d) show the liver somatic. Graphs (e) and (f) show condition factor. Graphs (g) and (h) show fork length. Comparisons are within seasons, and the same letters indicate no significant difference.

not consistently correspond with the elevated sediment retene. Brook stickleback from the two shallow lake environments were reproductively advanced, had greater condition factor, and larger livers than fish from the more riverine environment, suggesting that responses in fish are related to warmer water temperatures due to effluent load

Downloaded from http://iwaponline.com/wqrj/article-pdf/55/1/52/709313/wqrjc0550052.pdf
by guest

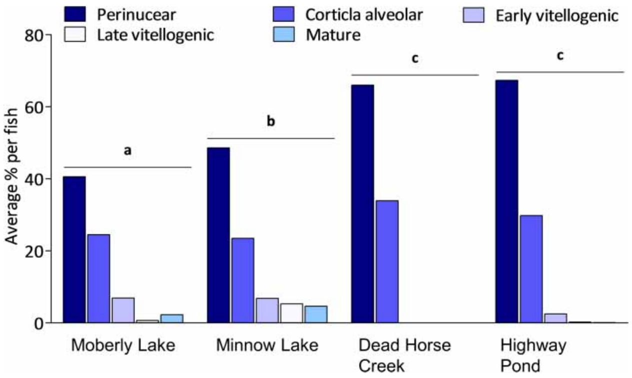

Figure 6 | Gonadal development of female brook stickleback captured in May 2011. Site groups with common subscripts are not significantly different.

in a low flow system rather than the chemical makeup of the effluent. The only consistent response in fish from the effluent-receiving environment was the greater liver size. The latter part of the study documented a complete collapse of the Moberly Lake brook stickleback population that followed a summer hypoxic period.

Retene is present in the sediment of Moberly Lake at levels approaching the highest reported in any receiving environment and CYP1A was inconsistent with this contamination. At over 1,600 μ/g dw, retene levels in Moberly Lake in 2007 are within the range of values (18.4–3,300 μg/g dw) previously reported at comparably contaminated sites (Leppänen & Oikari 1999a, 1999b, 2001; Lahdelma & Oikari 2005). Under anaerobic conditions, abietene resin acids are almost completely converted to retene (Tavendale *et al.* 1997a, 1997b), and in Moberly Lake sediment, they are 91% converted to neutrals. Sedimentary retene has been demonstrated as bioavailable in other circumstances (Leppänen & Oikari 1999a, 1999b; Oikari *et al.* 2003). The CYP1A-inducing ability of retene has been very well established in laboratory studies (Fragoso *et al.* 1998; Billiard *et al.* 1999; Brinkworth *et al.* 2003). From 1990 to 2000, Jackfish Bay white sucker showed significantly higher EROD levels compared with Mountain Bay (reference) white sucker (Bowron *et al.* 2009). Throughout 1990–2000, these differences declined steadily but remained significant (Bowron *et al.* 2009). Male white sucker captured in September 2000 still showed eight-fold EROD induction when compared with the reference location (Bowron *et al.* 2009). The directionally opposite response in male stickleback sampled in 2007 could be due to a refractory CYP1A phenotype in the exposed brook stickleback population. In a study by Meyer *et al.* (2002), killifish in a contaminated site showed a refractory CYP1A phenotype (decreased EROD activity). However, CYP1A response can also be altered during periods of reproductive development, which were variable across sampling years.

The patterns in somatic indices seen in Moberly Lake brook stickleback were inconsistent with the national response pattern of fish populations exposed to pulp and paper mill effluent. This pattern, observed in meta-analysis of data collected under the auspices of the EEM program, has been termed 'metabolic disruption' (Munkittrick *et al.* 1991). The pattern is characterized by higher condition factor and liver size, reflecting a more nutrient-enriched environment, but lower relative gonad size, reflecting an inability to metabolize stored energy into reproductive tissue. This response profile has been consistently observed in white sucker from Jackfish Bay between 1988 and 2006 with trends towards recovery.

Endpoints relative to reproduction were generally opposite that observed in both Jackfish Bay white sucker and in locally relevant fish at other pulp and paper mills in the past. The larger gonads in fish from the lake environments, coupled with much higher proportions of developed ovarian follicles, are due to differences in reproductive timing. Stickleback species undergo much of their gonadal development in the period just prior to spawning when compared with the large-bodied synchronous spawning white sucker that undergoes most of gonadal recrudescence in the fall and hold the follicles until final maturation in spring. The timing of reproductive development of monitoring species can substantially influence the conclusions. For example, rainbow darter (*Etheostoma caeruleum*) exposed to municipal sewage effluent demonstrated a different pattern of response in the spring than in the fall (Fuzzen *et al.* 2016). Brook stickleback exposed to municipal wastewater also demonstrated varying patterns of response between reference and exposed sites dependent on the month they were sampled (Tetreault *et al.* 2012). In that study, the May/June reproductive period was when the largest differences were exhibited between sampling locations although this also appeared to be a result of reproductive timing as was likely in the study described herein. The rapid gonad development of stickleback species makes it difficult to compare

Downloaded from http://iwaponline.com/wqrj/article-pdf/55/1/52/709313/wqrjc0550052.pdf
by guest

the relative energy put into reproductive tissue as vitellogenesis and egg maturation are occurring simultaneously within a short period. The nature of the reproductive behaviour of the two species may also impact the energetics of gonadal development. Sucker do not prepare or build and guard nests as stickleback species and as such may put more energy into gonadal tissue as eggs and fry may have a lower probability of survival when compared with species that have greater parental investment and care.

Temperature differences among sites throughout the year could explain the variable reproductive development between sites. Because of warmer temperatures during winter and into spring, fish living in a shallow lake environment matured earlier (Minnow Lake and Moberly Lake) than those in colder riverine environments, given that warmer waters play a major role in inducing maturation. In 2011, Moberly Lake was the warmest of the sites at spawning time. The presence of effluent appears to warm this lake by as much as 5° during the remainder of the year. These observations suggest that, in the winter prior to the 2011 spawning season, Moberly Lake's temperature was warm enough to encourage earlier reproductive development in brook stickleback. It could certainly be suggested that sampling brook stickleback in the spring is not ideal; however, unlike the white sucker, this species may not have a stable post-vitellogenic sampling window where fish gonad size is temporally stable, making brook stickleback less suitable for monitoring purposes (Barrett & Munkittrick 2010).

Changes in mill operation and treatment/processing of the effluent can improve effluent quality and reduce potency in its ability to induce reproductive changes in fishes. Brook stickleback were exposed to effluent for 6 and 8 months prior to the 2007 and 2011 sampling periods, respectively, in addition to being exposed to contamination in the sediment as described herein. White sucker captured in Lake Superior showed the strongest evidence of improved reproductive development during periods of mill closure (Bowron *et al.* 2009). In a laboratory study by Rickwood *et al.* (2006), exposure to 100% effluent from the Terrace Bay mill had no effect on gonad size of fathead minnow. While the number of spawning events was decreased, total egg production was not (Rickwood *et al.* 2006). In addition to the complexity of comparing different species between laboratory and dissimilar receiving environments, pulp mill effluent can vary temporally depending on current mill operations or treatment system upsets (Martel *et al.* 2011).

Condition factor in short-lived fishes may be more influenced by life history than food availability. There was no consistent effect of effluent exposure on condition factor as has been observed for in white sucker for the two decades from 1988 to 2007 (Bowron *et al.* 2009). Brook stickleback are thought to live for a single year or slightly longer (Acere & Lindsey 1986; King & Cone 2008). During this time, all available energy would go into growth in order to best maximize fecundity. Contrast this with a large-bodied, long-lived species such as the white sucker where the largest part of growth is completed in 3–4 years prior to maturation, after which growth gradually slows. Such species put proportionally less energy into growth; therefore, condition factor may change more dramatically in response to increased resource availability as may occur downstream of a pulp and paper mill effluent discharge (Environment Canada 2012, 2014). However, as the present study did not examine food availability, the influence of such cannot be ruled out here.

The liver size of brook stickleback from the effluent-receiving environment was clearly elevated compared with fish from reference sites. Minnow Lake and Moberly Lake females being further along in their maturity likely had engorged livers due to increased production of vitellogenin. However, the liver size in males is less confounded by reproductive development, and Moberly Lake males had consistently larger livers. Larger liver sizes in effluent-exposed fish when compared with unexposed fish is consistent with exposure to pulp mills in general (Munkittrick *et al.* 1994) and was observed in Jackfish Bay white sucker (1988–1993 females, 1988–2000 males), but inconsistent with the more recent trends in Jackfish Bay white sucker (1995–2006 females, 2002–2006 males; Bowron *et al.* 2009). In more recent years, there were no discernible differences in the relative liver sizes between Jackfish Bay white sucker and Mountain Bay white sucker. Unlike condition factor, liver size can respond to both increased energy intake (van den Heuvel *et al.* 2008) and the increased metabolic burden of contaminants including PAHs (Phalen *et al.* 2014).

The anoxia that occurred in Moberly Lake is the most probable reason for the eradication of brook stickleback. DO levels in Moberly Lake (0.7 mg/L and 7%) were lower

Downloaded from http://iwaponline.com/wqrj/article-pdf/55/1/52/709313/wqrjc0550052.pdf
by guest

than LC50 values for oxygen reported in Landman *et al.* (2005) for a range of both sensitive and resilient fish species. In that study, six of seven species had LC50 higher than 0.7 mg/L, and only the short-finned eel *Anguilla australis* had an LC50 lower than 0.7 (0.54 mg/L). The counterpoint to the hypothesis of hypoxia-related disappearance was the presence of an unidentified cyprinid species in 2012 and 2013 (although it was also absent in 2011). One possibility is that this species had a refuge from hypoxia not utilized by brook stickleback. There was a short ~10 m fast-moving section of stream at the downstream end of Moberly Lake prior to a steep ~15 m elevation drop that may have provided sufficient oxygenation to act as a refuge.

The combination of high temperature and large amounts of effluent passing through with a high BOD would have significantly reduced the amount of oxygen available. The furthest upstream portion of Moberly Lake is often observed to bubble, likely due to the significant amount of anaerobic bacterial activity. This is not the first time fish have disappeared from effluent-exposed areas in this basin. Before secondary treatment was installed in 1989, all populations of fish in Blackbird Creek were completely eliminated due to the effluent's acute toxicity (JBRAP 1991). Minnow trapping in 2007 by the investigators found brook stickleback at a number of locations throughout the creek. Since 1989, the addition of secondary treatment facilities monthly LC50 tests conducted as required by the regulatory regime indicate that the effluent from the outflow pipe is no longer acutely toxic (JBRAP 1991). However, a toxicity event from a spill or treatment failure cannot be ruled out.

Brook stickleback were able to thrive in a fairly harsh environment of both pulp mill effluent and severe historic sediment contamination. This species is well known to utilize beaver impoundments habitats, warm, organically enriched environments not dissimilar to the pulp mill-receiving environment that may be subject to periodic hypoxia. While there is no published experimental data for brook stickleback hypoxia tolerance, it might be assumed that they are relatively insensitive to hypoxia due to the nature of the environments they inhabit, and thus they can also inhabit shallow boreal forest lakes where few predators can survive (and, in fact, they are seldom found in any abundance where those fish predators exist). These characteristics, together with a short-lived life history, may make them less responsive to the types of physiological changes that manifest in larger longer-lived fishes in response to anthropogenic stress. While much work has been done on sublethal impacts of contaminants, particularly on reproduction, it is somewhat telling in this case that the local population may most likely have been eliminated by the acute effects of oxygen deprivation.

ACKNOWLEDGEMENTS

This study was financially supported by an NSERC Strategic Projects Grant to Dr. Kelly Munkittrick, and a Canada Research Chair held by Michael R. van den Heuvel. The authors wish to acknowledge Brad Scott, Travis James, Christina Pater, Colin Arens, Liane Leclair, Tim Arciszewski, Gerald Tetrault, and Mark McMaster for their assistance in the field.

REFERENCES

Acere, T. O. & Lindsey, C. C. 1986 Age, growth, and life history of *Culaea inconstans* (Piscese: Gasterostidae) in Delta Marsh Lake Manitoba. *Hydrobiologia* **135**, 35–44.

Barrett, T. & Munkittrick, K. 2010 Seasonal reproductive patterns and recommended sampling times for sentinel fish species used in Environmental Effects Monitoring programs in Canada. *Environmental Reviews* **18**, 115–135.

Barrett, T., Lowell, R. B., Tingley, M. A. & Munkittrick, K. R. 2010 Effects of pulp and paper mill effluent on fish: a temporal assessment of fish health across sampling cycles. *Environmental Toxicology and Chemistry* **29**, 440–452.

Basu, N., Ta, C. A., Waye, A., Mao, J., Hewitt, M., Arnason, J. T. & Trudeau, V. L. 2009 Pulp and paper mill effluents contain neuroactive substances that potentially disrupt neuroendocrine control of fish reproduction. *Environmental Science & Technology* **43**, 1635–1641.

Billiard, S. M., Querback, K. & Hodson, P. V. 1999 Toxicity of retene to early life stages of two freshwater fish species. *Environmental Toxicology and Chemistry* **18**, 2070–2077.

Bowron, L. K., Munkittrick, K. R., McMaster, M. E., Tetreault, G. & Hewitt, L. M. 2009 Responses of white sucker (*Catostomus commersoni*) to 20 years of process and waste treatment changes at a bleached kraft pulp mill, and to mill shutdown. *Aquatic Toxicology* **95**, 117–132.

Brinkworth, L. C., Hodson, P. V., Tabash, S. & Lee, P. 2003 CYP1A induction and blue sac disease in early developmental stages of rainbow trout (*Oncorhychus mykiss*)

Downloaded from http://iwaponline.com/wqrj/article-pdf/55/1/52/709313/wqrjc0550052.pdf
by guest

exposed to retene. *Journal of Toxicology and Environmental Health A* **66**, 627–646.

Environment Canada 2012 *Fifth National Assessment of Environmental Effects Monitoring Data From Pulp and Paper Mills Subject to the Pulp and Paper Effluent Regulations*, p. 27. Available from: http://publications.gc.ca/site/eng/436937/publication.html.

Environment Canada 2014 *Sixth National Assessment of Environmental Effects Monitoring Data From Pulp and Paper Mills Subject to the Pulp and Paper Effluent Regulations*, p. 25. Available from: https://www.canada.ca/en/environment-climate-change/services/managing-pollution/environmental-effects-monitoring/sixth-national-assessment-pulp-paper-mills.html.

Fragoso, N. M., Parrott, J. L., Hahn, M. E. & Hodson, P. 1998 Chronic retene exposure causes sustained induction of CYP1A activity and protein in rainbow trout (*Oncorhynchus mykiss*). *Environmental Toxicology and Chemistry* **17**, 2347–2353.

Fuzzen, M. L. M., Bragg, L. M., Tetreault, G. R., Bahamonde, P. A., Tanna, R. N., Bennett, C. J., McMaster, M. E. & Servos, M. R. 2016 An assessment of the spatial and temporal variability of biological responses to municipal wastewater effluent in rainbow darter (*Etheostoma caeruleum*) collected along an Urban Gradient. *PLoS ONE* **11**, e0164879.

Jackfish Bay Remedial Action Plan Team (JBRAP) 1991 *Stage 1: Environmental Conditions and Problem Definition*. North Shore of Lake Superior Remedial Action Plans, Terrace Bay, Ontario, p. 157. Available from: https://archive.org/stream/jackfishbayareao26016#page/n34/mode/1up.

Jackfish Bay Remedial Action Plan (JBRAP) Team 1998 *Stage 2: Remedial Strategies for Ecosystem Restoration*. North Shore of Lake Superior Remedial Action Plans, Terrace Bay, Ontario, p. 57.

Hewitt, M., Kovacs, T., Dube, M., MacLatchy, D., Martel, P., McMaster, M., Paice, M., Parrott, J., van den Heuvel, M. R. & Van Der, K. G. J. 2008 Altered reproduction in fish exposed to pulp and paper mill effluents: a review of the roles of individual compounds and mill operating conditions. *Environmental Toxicology and Chemistry* **27**, 682–697.

King, S. D. & Cone, D. K. 2008 Persistence of *Dactylogyruseucalius* (Monogenea: Dactylogyridae) on the Short-Lived Host *Culaea inconstans* (Pisces: Gasterosteiformes). *Journal of Parasitology* **94**, 973–975.

Kovacs, T., Martel, P., O'Connor, B., Parrott, J., McMaster, M., Van Der Kraak, G. J., MacLatchy, D., van den Heuvel, M. R. & Hewitt, L. M. 2011 Kraft mill effluent survey: progress toward best management practices for reducing effects on fish reproduction. *Environmental Toxicology and Chemistry* **30**, 1421–1429.

Kovacs, T., Martel, P., O'Connor, B., Hewitt, M., Parrott, J., McMaster, M., MacLatchy, D., Van Der Kraak, G. & van den Heuvel, M. R. 2013 A survey of Canadian mechanical pulp and paper mill effluents: insights concerning the potential to affect fish reproduction. *Journal of Environmental Science and Health A* **48**, 1178–1189.

Lahdelma, I. & Oikari, A. 2005 Resin acids and retene in sediments adjacent to pulp and paper industries. *Journal of Soils and Sediment* **5**, 74–81.

Landman, M. J., van den Heuvel, M. R. & Ling, N. 2005 Relative sensitivities of common freshwater fish and invertebrates to acute hypoxia. *New Zealand Journal of Marine and Freshwater Research* **39**, 1061–1067.

Leppänen, H. & Oikari, A. 1999a The occurrence and bioavailability of retene and resin acids in sediments of a lake receiving BKME (Bleached Kraft Mill Effluent). *Water Science and Technology* **40**, 131–138.

Leppänen, H. & Oikari, A. 1999b Occurrence of retene and resin acids in sediments and fish bile from a lake receiving pulp and paper mill effluent. *Environmental Toxicology and Chemistry* **18**, 1498–1505.

Leppänen, H. & Oikari, A. 2001 Retene and resin acid concentrations in sediment profiles of a lake recovering from exposure to pulp mill effluents. *Journal of Paleolimnology* **25**, 367–374.

Martel, P., O'Connor, B., Kovacs, T., Semeniuk, S., Hewitt, L. M., MacLatchy, D., McMaster, M., Parrott, J., van den Heuvel, M. R. & Van Der Kraak, G. J. 2011 Effluent monitoring at a bleached kraft mill: directions for best management practices for eliminating effects on fish reproduction. *Journal of Environmental Science and Health A* **46**, 833–843.

McMaster, M. E., Parrott, J. L. & Hewitt, L. M. 2006 A decade of research on the environmental impacts of pulp and paper mill effluent in Canada: field studies and mechanistic research. *Journal of Toxicology and Environmental Health B* **9**, 319–339.

Meyer, J. N., Nacci, D. E. & Di Giulio, R. T. 2002 Cytochrome p4501a (CYP1A) in killifish (*Fundulus heteroclitus*): heritability of altered expression and relationship to survival in contaminated sediments. *Toxicological Sciences* **68**, 69–81.

Munkittrick, K. R., Portt, C. B., Van Der Kraak, G. J., Smith, I. R. & Rokosh, D. A. 1991 Impact of bleached kraft mill effluent on population characteristics, liver MFO activity, and serum steroid levels of a Lake Superior white sucker (*Catostomus commersoni*) population. *Canadian Journal of Fisheries and Aquatic Sciences* **48**, 1371–1380.

Munkittrick, K. R., Van Der Kraak, G. J., McMaster, M. E. & Portt, C. B. 1992 Response of hepatic MFO activity and plasma sex steroids to secondary treatment of bleached kraft pulp mill effluent and mill shutdown. *Environmental Toxicology and Chemistry* **11**, 1427–1439.

Munkittrick, K. R., Van Der Kraak, G. J., McMaster, M. E., Portt, C. B., van den Heuvel, M. R. & Servos, M. R. 1994 Survey of receiving water environmental impacts associated with discharges from pulp mills. 2. Gonad size, liver size, hepatic EROD activity and plasma sex steroid levels in white sucker. *Environmental Toxicology and Chemistry* **13**, 1089–1101. https://doi.org/10.1002/etc.5620130710.

Downloaded from http://iwaponline.com/wqrj/article-pdf/55/1/52/709313/wqrjc0550052.pdf
by guest

Oikari, A., Fragoso, N., Leppänen, H., Chan, T. & Hodson, P. V. 2003 Bioavailability to juvenile rainbow trout (*Oncorhychus mykiss*) of retene and other mixed function oxygenase-active compounds from sediments. *Environmental Toxicology and Chemistry* **21**, 121–128.

Phalen, L. J., Köllner, B., Hogan, N. S. & van den Heuvel, M. R. 2014 The effects of benzo[a]pyrene on leukocyte distribution and antibody response in rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology* **147**, 121–128.

Rämänen, H., Lassila, H., Lensu, A., Lahti, M. & Oikari, A. 2010 Dissolution and spatial distribution of resin acids and retene in sediments contaminated by pulp and paper industry. *Journal of Soils and Sediment* **10**, 349–358.

Rickwood, C. J., Dubé, M. G., Hewitt, L. M., Kovacs, T. G. & MacLatchy, D. L. 2006 Use of paired fathead minnow (*Pimphales promelas*) reproductive test. Part 2: source identification of biological effects at a bleached kraft pulp mill. *Environmental Toxicology and Chemistry* **25**, 1847–1856.

Sandström, O. & Neuman, E. 2003 Long-term development in a Baltic fish community exposed to bleached pulp mill effluent. *Aquatic Ecology* **37**, 267–276.

Sherman, K., Clement, R. E. & Tashiro, C. 1990 The distribution of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in Jackfish Bay, Lake Superior, in relation to a kraft pulp mill effluent. *Chemosphere* **20**, 1641–1648.

Tavendale, M. H., McFarlane, P. N. & Mackie, K. L. 1997a The fate of resin acids-1. The biotransformation and degradation of deuterium labelled dehydroabietic acid in anaerobic sediments. *Chemosphere* **35**, 2137–2151.

Tavendale, M. H., McFarlane, P. N. & Mackie, K. L. 1997b The fate of resin acids-2. The fate of resin acids and resin acid derived neutral compounds in anaerobic sediments. *Chemosphere* **35**, 2153–2166.

Tetreault, G. R., Bennett, C. J., Cheng, C., Servos, M. R. & McMaster, M. E. 2012 Reproductive and histopathological effects in wild fish inhabiting an effluent-dominated stream, Wascana Creek, SK, Canada. *Aquatic Toxicity* 110–111. doi:10.1016/j.aquatox.2012.01.004.

United States Environmental Protection Agency (USEPA) 2006 *Histopathology Guidelines for the Fathead Minnow (Pimephales promelas) 21-day Reproduction Assay*, p. 53. Available from: nepis.epa.gov/Exe/ZyPDF.cgi/P100N3BN.PDF?Dockey=P100N3BN.PDF.

van den Heuvel, M. R. 2010 Recent progress in understanding the causes of endocrine disruption related to pulp and paper effluents. *Water Quality Research Journal of Canada* **45**, 137–144.

van den Heuvel, M. R., Dixon, D. G., Munkittrick, K. R. & Stegeman, J. J. 1995 Second round interlaboratory comparison of hepatic ethoxyresorufin-*O*-deethylase activity in white sucker (*Catostomus commersoni*) exposed to bleached-kraft pulp mill effluent. *Environmental Toxicology and Chemistry* **14**, 1513–1520.

van den Heuvel, M. R., Landman, M. J., Finley, M. A. & West, D. W. 2008 Altered physiology of rainbow trout in response to modified energy intake combined with pulp and paper effluent exposure. *Ecotoxicology and Environmental Safety* **69**, 187–198.

van den Heuvel, M. R., Slade, A. H. & Landman, M. J. 2010 Summary of a decade of research on the effects of a New Zealand pulp and paper mill on reproduction in fishes. *Water Quality Research Journal of Canada* **45**, 123–135.

First received 25 February 2019; accepted in revised form 18 June 2019. Available online 15 July 2019

Downloaded from http://iwaponline.com/wqrj/article-pdf/55/1/52/709313/wqrjc0550052.pdf
by guest