Environmental Conservation (2015) **42** (2): 152–161 © Foundation for Environmental Conservation 2014 doi:10.1017/S0376892914000319

Potential risks of trophic impacts by escaped transgenic salmon in marine environments

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Date submitted: 30 October 2013; Date accepted: 1 August 2014; First published online 15 September 2014

SUMMARY

There is significant concern about potential ecological effects of introduced organisms, including non-indigenous species and those created by genetic modification. This paper presents an Ecopath with Ecosim modelling approach, designed to examine long-term trophic effects of growth hormone (GH) transgenic coho salmon should they ever escape to a coastal salmonid ecosystem, namely the Strait of Georgia in British Columbia (Canada). The model showed that the effects of introduced GH transgenic coho salmon varied with their biomass, diet, structure of the invaded ecosystem, and environmental conditions. Occasional escapes of non-reproductive salmon did not have a significant impact on the example ecosystem. However, effects of GH coho salmon varied with their diet when large numbers of these fish were present in the simulated ecosystem (for example, when they constituted 20% of total current aquaculture production in the area). Further, climate-driven changes in the biomass of low trophic levels (bottom-up effects) could have a greater impact on the ecosystem than the introduction of large numbers of GH coho salmon. A new version of Ecopath with Ecosim's Monte Carlo approach showed that the model predictions were robust to GH coho salmon's Ecopath parameters, but more sensitive to vulnerabilities of prey to GH coho salmon. Modelling ecosystem effects of genetically modified organisms provides a complementary approach for risk assessments when data from nature are not readily obtainable.

Keywords: bottom-up effects, Ecopath with Ecosim, ecosystem, growth hormone, Monte Carlo simulation, salmon, transgenic, trophic interactions, vulnerabilities

INTRODUCTION

Significant concerns are associated with potential ecological effects of introduced organisms, including non-indigenous species and those created by genetic modification. Records of introductions based on reports in FishBase indicate that 1424 freshwater fish introductions have been transferred from one country to another, 64% of which have become established (Ruesink 2005). There has been a dramatic increase in the rates at which exotic species, introduced by ballast water in ships, are becoming established in ports worldwide (Bax *et al.* 2003), and climatic changes provide new opportunities for the spread of non-native species (Cheung *et al.* 2009; Walther *et al.* 2009). However, the role of invasive species remains unknown in a more integrated context involving changes in community and ecosystem structure (Walther *et al.* 2009). Specific phenotypic characteristics of introduced species play key roles in their success as invaders (Kolar & Lodge 2002) by introducing novel ecological capabilities into ecosystems. Most introduced organisms are derived from other geographies where their range, tolerance to abiotic and biotic factors, and interactions with other ecosystem components are known and such data may be applied to new scenarios to predict potential impacts. However, while no data from nature exist for genetically-engineered organisms, estimates of potential impacts on natural ecosystems are required. These animals can only be studied in confined laboratory facilities.

We focus on growth-hormone (GH) transgenic fish, a special type of engineered organism that has the potential to enter natural ecosystems in the future. Since the first transgenic fish were produced in China (Zhu *et al.* 1985), there have been more than 30 species of genetically-engineered fish, including many of the major world aquaculture species (Devlin *et al.* 2006). GH transgenic fish can grow substantially faster than their unmodified counterparts: for example, GH transgenic coho salmon (*Oncorhynchus kisutch*, hereafter GH coho) are on average 11 times larger than non-transgenic coho salmon at 14 months of age (Devlin *et al.* 1994, 2004*a*). Recently, genetically engineered, or transgenic, strains of fish have been considered for use in aquaculture, and risk assessments are underway to evaluate effects should such fish accidentally enter natural ecosystems.

Previous modelling studies involving transgenic fish have focused on fitness effects and transgene frequencies in populations (Davis *et al.* 1999; Muir & Howard 1999; Valosaari *et al.* 2008; Ahrens & Devlin 2011) while the resilience of and consequences for the receiving ecosystems have not yet been modelled. Sterilization could eliminate or reduce propagation of transgenes arising from breeding in nature

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Supplementary material: available online at http://dx.doi.org/10.1017/S0376892914000319

(Devlin & Donaldson 1992). However, even with a 100% sterilization success rate, the ecosystem may still be impacted for the lifespan of escapees, because GH transgenic fish are vigorous foragers and competitors for prey when compared to the wild type (Devlin *et al.* 1999; Sundström *et al.* 2004). Furthermore, to date and to the best of our knowledge, no quantitative studies have predicted the trophic effects on the whole ecosystem before the entry of transgenic fish.

Here we apply complex ecosystem modelling to an example ecosystem in the Strait of Georgia (SoG), British Columbia (BC). Modelling the potential influences of genetically-modified fish under different scenarios provides estimates that cannot otherwise be obtained through empirical experimentation in nature. Ecosystem modelling has been widely used to study ecosystem function and make predictions (Perry *et al.* 2010). Alien species have been successfully incorporated into Ecopath with Ecosim (EwE) models to simulate historic invasions (Harvey & Kareiva 2005; Espinosa-Romero *et al.* 2011; Langseth *et al.* 2012). Here we apply a recently published EwE model for the SoG marine ecosystem (Li *et al.* 2010) to examine the potential influence of escaped GH coho in the SoG on the whole food web in the context of environmental variability and fishing. Whereas the majority of salmon farming communities have not endorsed, and none are using, transgenic fish for aquaculture at this time, the SoG is an ideal candidate model ecosystem to investigate ecological impacts of GH coho because it is within the species' native range and substantial information exists on the functioning of the SoG ecosystem.

We examined a hypothetical example scenario that concentrated on the functioning of the SoG marine ecosystem to escaped GH coho and evaluated the potential long-term changes. Unlike many invasion biological studies trying to predict the establishment success of alien species, we added varying biomass levels of GH coho to the SoG marine ecosystem model and examined the consequences of trophic interactions on the organisms within this ecosystem. We focused on their marine life as sterilized GH fish for the purpose of simplification (we assumed no reproduction). Our aim was not to carry out accurate and precise predictions of changes of biomass of ecosystem members, but rather to detect general trends and the most sensitive trophic linkages, and to identify important impact factors influencing trophic interactions. We also consider a wide range of values for each GH coho parameter to assess the uncertainty of our predictions.

METHODS

Ecopath with Ecosim parameterization

We added a GH coho group to an existing SoG Ecopath model (Li et al. 2010) to assess potential effects at the ecosystem level. Detailed methodology and parameterization values for the GH coho are provided (supplementary material). In addition to applying the same diet as for resident salmon, we also defined three additional diets for GH coho: specifically a forage diet, forage hake diet, and broad diet, assuming that the contributions of selected prey are proportional to their biomass (see Fig. 1 for diet compositions). With regards to predation, we assumed that GH coho share the resident salmon's contribution, but do not necessarily contribute the same proportion, depending on their size and vulnerability, to the diets of all of their predators. We also assumed that GH coho have the same fishing mortality as the resident salmon. In summary, in the Ecopath model, GH coho and resident salmon have different production rates (production/biomass, P/B), consumption rates (consumption/biomass, Q/B), predation mortality, and potentially diets.

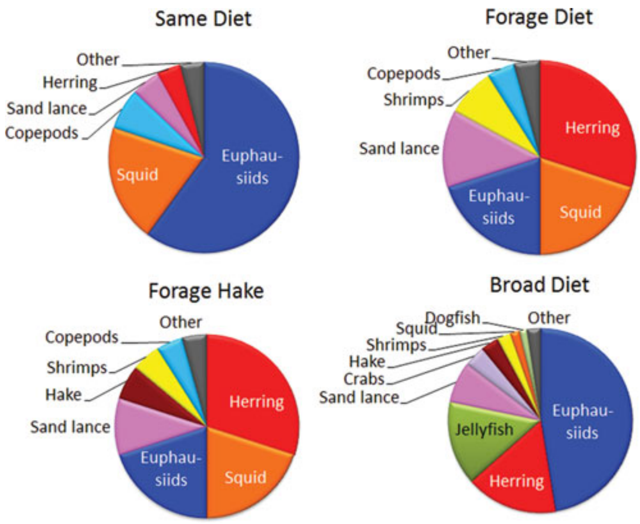


Figure 1 In addition to the same diet as resident salmon (Same Diet), three additional potential diets for growth-hormone transgenic coho salmon were modelled in Ecopath involving different prey: forage fish and invertebrates (forage diet), forage diet plus hake (forage hake diet), and all animals they encounter (broad diet). We also assume that prey contribution is proportional to their biomass.

Vulnerabilities are key parameters in Ecosim, representing the degree to which a large increase in predator biomass will cause predation mortality for a given prey based on foraging arena theory (Christensen *et al.* 2008; Ahrens *et al.* 2012). A low vulnerability (1 < vulnerability < 2) determines that how much the predator can feed on a prey species is controlled by the prey and not the predator, where an increase in predator biomass will not cause any significant increase in the predation mortality on the given prey (so-called bottom-up control). In contrast, a higher vulnerability (vulnerability > 2) indicates the opposite (top-down control), such that if the predator biomass is doubled, it will come close to doubling the predation mortality (Christensen *et al.* 2008). The same vulnerabilities, scaling to the prey trophic levels, were applied as in Li *et al.* (2010) for all groups. The GH coho vulnerabilities to predators were set the same as the wild population at 9.9 (top down). The prey vulnerabilities to GH coho value were set to the default value of 2 (intermediate) and adjusted to a wide range of vulnerabilities in the uncertainty analyses. Prey vulnerabilities to resident salmon were kept > 2 (greater than the prey vulnerabilities to GH coho) because GH coho have inferior swimming abilities (Farrell *et al.* 1997; Lee *et al.* 2003) that would likely impair their abilities to predate.

Ecopath with Ecosim scenarios

We simulated two major types of scenarios using Ecopath with Ecosim (EwE) version 6, running each for a 30-year period (the model runs 12 time steps per simulated year). The first series of scenarios (Table 1, Scenarios U1–U3, F1–F3), under fixed ocean conditions (no changes in primary and secondary production caused by ocean conditions were applied), compared the effects of (1) resident salmon and GH coho at similar levels with the same diet, (2) GH coho diets, and (3) GH coho with the same diet at different biomass levels. In the second set of scenarios (Table 1, Scenarios F4–F8), we added climate change effects by changing the biomass of lower trophic levels such as those of euphausiids and herring. Euphausiids and other zooplankton have experienced large climate-caused changes in biomass over the last few decades in the SoG (Li *et al.* 2013; Mackas *et al.* 2013). For both types of scenarios, a forcing function was used to maintain the biomass of salmon (GH coho or resident) and/or other groups (such as euphausiids) at a fixed level over 30 years.

For scenarios under fixed ocean conditions, we considered escapes at the current resident salmon level (Scenarios U1–U2, Table 1), at the historic average levels (Scenario U3, Table 1), and as large introductions that may arise under extreme conditions (for example as catastrophic escapes, but still without further reproduction, Scenarios F1–F3, Table 1). We first obtained historic SoG salmon farm production data from the BC Ministry of Agriculture (see http://www.env.gov.bc.ca/omfd/fishstats/index.html). We used the average annual total production of farmed salmon in the SoG (all species including Atlantic salmon *Salmo salar L.*, chinook salmon *Oncorhynchus tshawytscha*, coho salmon, and steelhead *Oncorhynchus mykiss*) from 2000 to 2010 as potential for a maximum total GH coho production in this area. In addition to average escape rate, we also considered the extreme storms. The escape of Atlantic salmon in Norway is dominated by structural failures of equipment caused by severe environmental events, and the environmental forcing has sometimes been so powerful as to completely break down a fish farm (Jensen *et al.* 2010). Weather extremes have increased and are expected to continue increasing in the future (Rahmstorf & Coumou 2011; Coumou & Rahmstorf 2012). Although extremely unlikely to ever occur in reality, using a very high biomass of introduced GH coho over a lengthy term approximates a maximal level of impact that could be anticipated from non-reproductive animals. For example, a biomass of 10 times of the Ecoapth baseline of resident salmon is equivalent to an introduction of approximately 20% of the total farmed salmon. To compare effects of GH coho and wild-type populations, we ran the modified model of Li *et al.* (2010) forcing the salmon population (GH coho or resident) of a constant level 10 times its initial biomass for 30 years (Scenarios F1 and F2, Table 1). We also used a biomass forcing function to hold GH coho biomass constant at 5, 15, 20 or 25 times its initial biomass for 30 simulated years (Scenario F3, Table 1).

For the scenarios exploring altered ocean conditions, we examined the responses of the functional groups to a combination of decreased euphausiids or herring and different GH coho or resident salmon conditions (Scenarios F4–F8, Table 1). A simulation was first run keeping euphausiid biomass 15% lower than its baseline combined with 10 times biomass forcing of salmon (GH coho or resident) (Scenarios F4 and F5, Table 1). We then held the same diet of GH coho at 10 times its baseline and kept euphausiid or herring biomass constant at –30%, –15%, +15%, or +30% that of the baseline level (Scenarios F6–F8, Table 1).

Model sensitivity

Considering the huge uncertainty associated with GH coho parameters based on currently available empirical data, we performed two separate analyses to examine the sensitivity of the initial Ecopath and Ecosim parameters. We completed the two sensitivity analyses by keeping the biomass of GH coho constant at 10 times that of the resident salmon and with the same diet (the same diet of Scenario F1), and compared the perturbed Ecosim predictions with the Ecosim baseline. However, the biomass forcing of GH coho may affect the influence of changes in production rate (production/biomass, P/B) on the GH coho population dynamics because this forcing function always keeps the population at a specific constant level, no matter how the production rate shapes the population. We thus repeated the two sensitivity analyses with no forcing on the GH coho, but with a fixed 15% reduction in euphausids (the same diet of Scenario F6).

For the Ecopath parameter sensitivity analyses, we used a Monte Carlo approach (Christensen & Walters 2004; Christensen *et al.* 2008). We first improved the Monte Carlo function in EwE 6 by adding consumption rate and a new parameter EET (ecotrophic efficiency tolerance) to the list of parameters that can be varied on the main Monte Carlo interface. We then set a small number (0.00005) for EET that allowed Ecopath models to run under close to a balanced state rather than requiring an exact balance (here $1 + \text{EET} > \text{EE} > 0 - \text{EET}$ instead of $1 > \text{EE} > 0$; see EE in supplementary material), thereby minimizing the difference between the range of the parameter values actually used and set by the user. We set a wide range (upper and lower limit) for consumption rate of GH coho salmon at 7.3–21.9, which is 1–3 times the value for resident salmon, based on experiments under laboratory conditions (Devlin *et al.* 1999, 2004*b*; Sundström *et al.* 2004, 2005). Similarly, we also consider a wide range 1.2–3.6 times that of the resident salmon, which is 2.6–7.9 for GH coho. Additionally, we set the coefficient of variation to a large value (for example 1000) to emulate a uniform distribution

Table 1 Summary of all scenarios' 30-year simulations based on the modified model of Li *et al.* (2010). No forcing was needed for the first three scenarios (U1–U3) because the biomass of GH coho remained at the level set as below automatically. The same diet of Scenarios 1 and 6 was used for model uncertainty analyses.

Scenario	*GH coho biomass*	*GH coho diet*	*Altered biomass of other groups*	*Figure*
U1	Present, no forcing	Four diets		
U2	Present, no forcing	Four diets	Resident salmon absent	
U3	Average escape	Four diets		
F1	10 times forcing	Four diets		2*a, b*
F2	Present, no forcing	Same diet	Resident salmon 10 times forcing	2*b*
F3	5,10,15,20, 25 times forcing	Same diet		2*c*
F4	10 times forcing	Four diets	Euphausiid forcing at –15% of its baseline	2*d*
F5	Present, no forcing	Same diet	Euphausiid forcing at –15% of its baseline; resident salmon 10 times forcing	2*d*
F6	Present, no forcing	Four diets	Euphausiid forcing at –15% of its baseline	2*e*
F7	10 times forcing	Same diet	Euphausiid forcing at –30%, +15% and +30% of its baseline	2*f*
F8	10 times forcing	Same diet	Herring forcing at –30%, –15%, +15% and +30% of its baseline	

within the upper and lower limit so that new sets of Ecopath parameters are randomly drawn within the range. Ecosim simulates the ecosystem only when a new balanced Ecopath model (here within EET) is achieved (Christensen & Walters 2004; Christensen *et al.* 2008). If ecotrophic efficiency for any group is greater than 1.0 + EET or less than 0 – EET, then the input parameters will be rejected and another attempt was made to vary the Ecopath parameters. We completed 200 successful Monte Carlo runs with altered production and consumption rates, single or combined, to compare with the Ecosim baseline.

For the Ecosim parameter sensitivity analysis, we varied the vulnerabilities related to GH coho in Ecosim. For the Ecosim baseline, the same vulnerabilities were applied as in Li *et al.* (2010) for all groups. The GH coho vulnerabilities to predators value was set the same as the wild population at 9.9 (top down) and the prey vulnerabilities to GH coho value was set to the default value of 2 (intermediate). We re-ran the same diet of Scenario F1 and Scenario F6 with varying vulnerabilities: GH coho vulnerabilities to their predators were set to 100 (strong top-down control on GH coho) or 1.5 (strong bottom-up control by GH coho), and prey vulnerabilities to GH coho reset to 100 or 1.5. We finally compared the Ecosim predictions using these four different vulnerability settings with the Ecosim baseline.

RESULTS

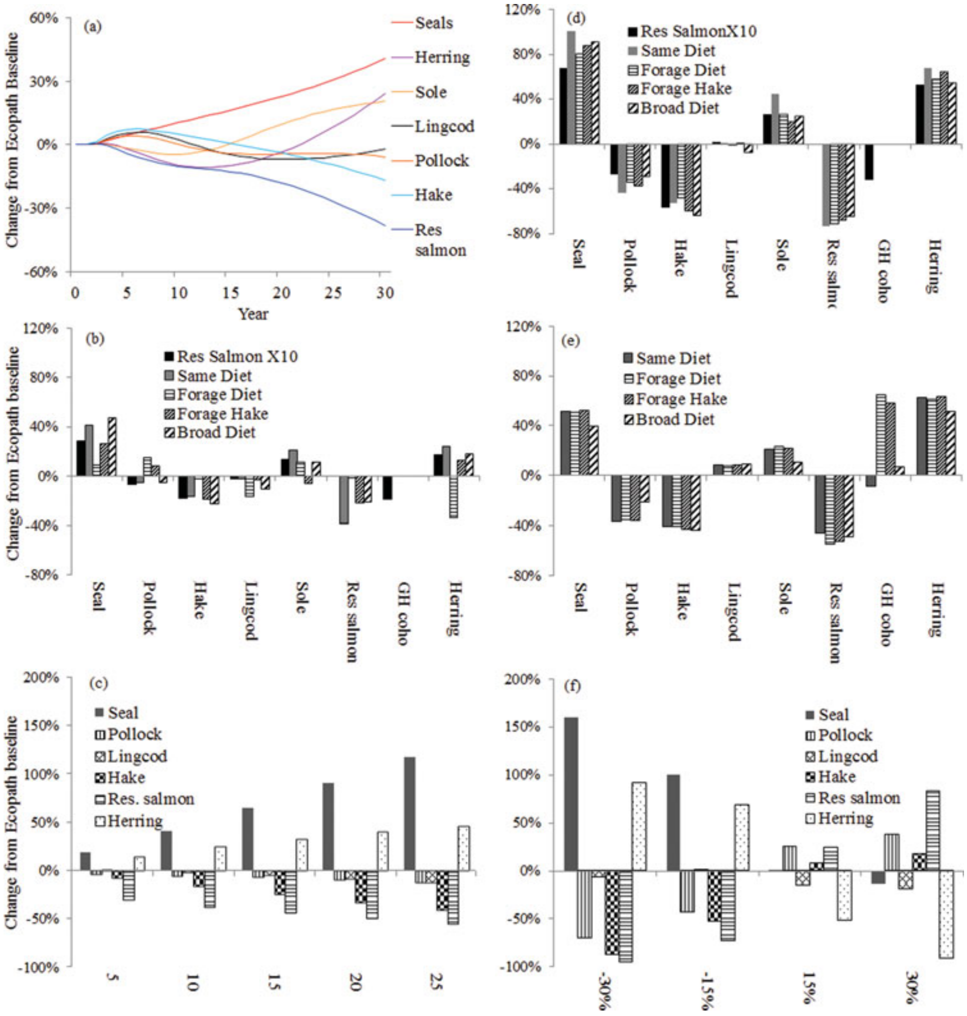
Model responses to increased biomass of salmon under fixed ocean conditions

The presence of GH coho either at the same biomass level as resident salmon, or at historic average escape level (Scenarios U1–U3, Table 1), had only minor impacts (< 1% in 30 years) on the model ecosystem components. Adding the same biomass of GH coho as resident salmon to the model allowed the Ecopath model to remain balanced and all groups stayed stable, suggesting resilience in the ecosystem against this scale of introduction. When resident salmon were completely replaced with the same biomass of GH coho, changes in species group biomasses were also very small (< 1% in 30 years). Based on the average escape rate of salmon from sea cages of 0.2% in Norway (Jensen *et al.* 2010), introduced GH coho would constitute at most 10% of the wild population, and their effects at this level also would not be large.

When the biomass of GH coho was forced to a level 10 times that of the resident salmon baseline (namely equivalent to a massive introduction representing 20% total farmed salmon in the region), with the same diet, effects on different groups changed considerably over time (the same diet of Scenario F1; Fig. 2*a*). Shortly after the introduction of GH coho, most groundfish increased due to elevated salmon biomass and hence increased contribution to seal diet. As a result, increased seal biomass in turn increased predation on all prey including hake that compete for food with GH coho (Fig. 2*a*) (see diet of harbour seal and other groups in Li *et al.* (2010)). Herring biomass eventually increased as a result of decreased predation by hake (their most significant predator). Resident salmon biomass decreased as a result of increased predation by harbour seals and competition for food with GH coho. This example showed effects over time, whereas for other scenarios below, only the final ecosystem condition after 30 years is presented.

When the biomass of resident (non-transgenic) salmon or GH coho were held to a level 10 times of their baseline, with the same diet as the resident salmon (Table 1, Scenarios F1 and F2), the long-term effects on functional groups were similar. In contrast, different responses were seen when GH coho foraged on four different diets (Fig. 2*b*). The difference in harbour seal between 10 times forcing of resident salmon and GH coho with the same diet, is due to the assumption that GH coho would share predation with resident salmon by seals, and contribute more to seals' diet than their counterpart at the same biomass. Thus, increased GH coho salmon with the

Figure 2 Predicted biomass of major functional groups relative to the Ecopath baseline (*a*) over the 30 years or (*b–f*) in the thirtieth year. (*a, b* and *c*) Under fixed ocean conditions: (*a*) when the growth-hormone transgenic (GH) coho salmon with the same diet as the resident salmon was forced to 10 times its baseline for 30 years, (*b*) when the biomass of resident salmon (black left hand bar) or GH coho salmon with dour diets (remaining bars) respectively was forced to 10 times its baseline for 30 years, and (*c*) when the biomass of GH coho, with the same diet as resident salmon, was forced to be 5, 10, 15, 20 and 25 times the baseline of resident salmon for 30 years. (*d*, *e* and *f*) Under altered ocean conditions: (*d*) when the biomass of GH coho or resident salmon was forced to remain10 times that of resident salmon baseline and when biomass of euphausiids was forced to decrease by 15% of the baseline, (*e*) with biomass of euphausiids was forced to decrease by 15% of the baseline only, (*f*) with euphausiid biomass was forced to be constant at four different levels: decreasing 30% and 15%, and increasing15% and 30% its baseline for 30 years, with forcing the biomass of GH coho, with the same diet,10 times its baseline. Note that forced biomass was not presented in the figures.



same diet in the model caused a greater increase in the seal and consequently increased predation on prey including resident salmon. Our model also showed that the changes in functional groups varied substantially with GH coho diets, and even had opposite trends with different diets (Fig. 2*b*). Resident salmon declined to the greatest degree when GH coho competed for the same diet, whereas only small changes to resident salmon were observed when GH coho had a forage diet. Herring biomass declined greatly when GH coho used a forage diet, and preyed heavily on herring. In contrast, herring biomass increased when GH coho used any of the other three diets. Under the forage hake diet, although herring also contributed a similar proportion to GH coho diet, GH coho also preyed on the herring's largest predator, hake, which thus reduced the predation from hake on herring. Hake declined the least under the forage diet, due to a minimally increased seal population. With any of the diets used by GH coho, the biomass of hake, resident salmon and lingcod decreased and seal biomass increased, while directional changes in the biomass of herring, sole and pollock depended on the diet of GH coho. Changes in the functional groups increased with greater biomass of GH coho with the same diet (Fig. 2*c*).

Model responses to altered ocean conditions

Reducing euphausiid biomass by 15% of the baseline in addition to 10 times forcing of GH coho or resident salmon (Scenarios F4 and F5, Table 1) generally produced much stronger effects (Fig. 2*d*) than the forcing of salmon alone (Fig. 2*b*). The biomass of hake, salmon and pollock greatly decreased due to the lower levels of its major prey (euphausiids) and higher predation by seals. Decreased pollock contributed greatly to an increase in sole biomass. Herring biomass increased due to reduced predation from hake, while herring acquired enough food from copepods. Seals benefited from increased herring prey and from the presence of GH coho. Different GH coho diets produced the same trends and no large difference in the responses of functional groups (Fig. 2*d*), again indicating the climate-driven bottom-up effects were more influential.

With decreased euphausiids (Scenario F6, Table 1), pure bottom-up effects were still stronger than 10 times forcing of salmon, and GH coho diet only had large effects on GH coho itself (Scenario F6, Table 1; Fig. 2*e*). GH coho biomass was low under the same diet and broad diet conditions where they fed heavily on euphausiids and were in strong competition with resident salmon. As prey vulnerabilities to resident salmon were higher than prey vulnerabilities to GH coho, resident salmon were more sensitive to the decrease of euphausiids. GH coho benefited from increased herring and therefore greatly increased under the forage diet and the forage and hake diet. Compared to the forcing of both euphausiids and salmon (Fig. 2*d*), decreased euphausiids resulted in the same trends (increases or decreases) among functional groups, but with smaller changes in some groups (Fig. 2*e*). For example, with no extra food in the form of GH coho, harbour seals increased less and thus hake declined less.

Altered euphausiids biomass, together with 10 times the GH coho with the same diet, affected the whole ecosystem greatly (Scenario F7, Fig. 2*f*). The biomass of resident salmon substantially varied from the increase or decrease of euphausiids biomass. The presence of more euphausiids resulted in more hake and pollock, which in turn increased predation on herring. Harbour seals suffered from a reduction of herring. Altering herring biomass was found to have inverse effects compared to varying euphausiid biomass. These responses corroborated strong bottom-up effects across trophic levels and a strong role of the harbour seal-hake-herring trophic triangle in ecosystem functioning.

Sensitivity analyses

For the same diet for Scenario F1, and forcing GH coho to 10 times the resident salmon baseline level, Ecosim estimates of biomass in functional groups were robust to production rate and consumption rate of GH coho (Fig. 3*a*). With variable production rate or consumption rate, the mean biomass of each functional-group was very close to the Ecosim baseline, with the maximum difference of 1.9% seen for seal biomass. However, the variability in predicted biomass was larger with variable production rate than with variable consumption rate, except for wild resident salmon which were impacted strongly by GH coho consumption rate. A combined effect also produced similar variability, with slightly larger deviation in the mean functional-group biomass than seen with production rate or consumption rate alone. This result was based on the initial vulnerability settings, and the consumption rate with a start value set in Ecopath may change over the 30-year ecosystem simulation.

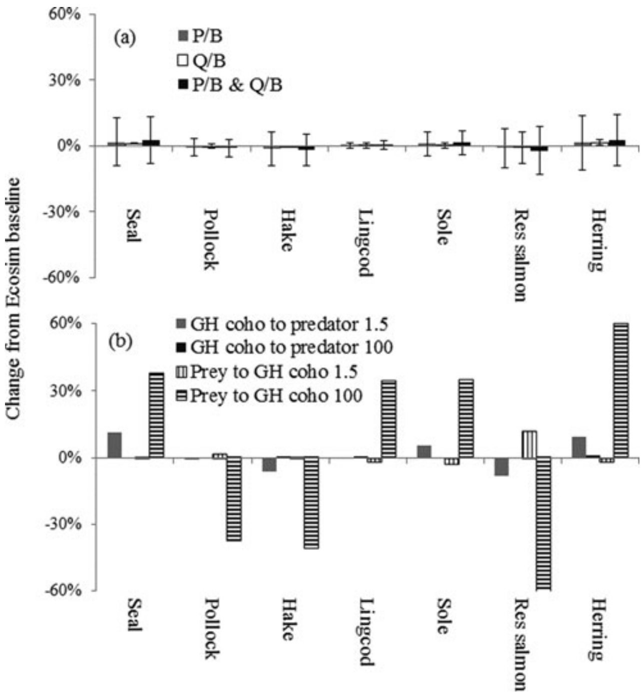


Figure 3 Results of uncertainty analyses keeping the biomass of growth-hormone transgenic (GH) coho with the same diet 10 times that of the resident salmon: (*a*) deviation of 200 Monte Carlo runs from the Ecosim baseline (mean ± standard deviation) with altering Ecopath parameters of GH coho: (1) uniform-distributed production rate (production/biomass, P/B, from 2.6–7.9); (2) uniform-distributed consumption rate (consumption/biomass, Q/B, from 7.3–21.9); and (3) combined P/B and Q/B parameters; (*b*) deviation of Ecosim output from baseline with varying predator-prey interactions at four levels: GH coho vulnerability to predators of 1.5 and 100, and the prey vulnerability to GH coho of 1.5 and 100. Vulnerabilities > 2 indicate top-down control from predators and < 2 indicate bottom-up control from prey. Note the difference of consumption rate set in Ecopath and actual rate in Ecosim (see Model uncertainty in Discussion).

Ecosim estimates of biomass in functional groups were more sensitive to the predator-prey interactions (Fig. 3*b*). Among the different vulnerabilities tested, prey vulnerability to GH coho of 100 (namely strong top-down control by GH coho) produced a greater difference from the baseline than the other three vulnerabilities (GH coho vulnerability to predators of 1.5 and 100, and prey vulnerability to GH coho of 1.5), and ranked top among all the parameters considered in the sensitivity analyses. Under this vulnerability setting, the average deviation of all groups was 44%, with the largest difference of 60% in resident salmon and herring (Fig. 3*b*), compared to the Ecosim baseline (Fig. 2*b*). Resident salmon were almost extirpated, with this scenario having the worst impact on resident salmon among all scenarios examined. The effects on the whole ecosystem were the largest when GH coho had top-down control on their prey.

For the same diet as in Scenario F6 (forcing a 15% decline in euphausiids with GH coho present but no forcing), varying GH coho Ecopath parameters had a minor influence on Ecosim estimates of biomass, probably due to the low biomass of GH coho. For the combined production rate and consumption rate, Ecosim estimates of biomass of GH coho had a mean deviation of 7%, and other groups < 2.6%. Unlike Scenario F1, setting prey vulnerabilities to GH coho of 100 (under the same diet of Scenario F6) only had the large effects on GH coho and resident salmon groups, again suggesting the weak effects of GH coho at low biomass. Under this vulnerability setting, GH coho declined by 73% and resident salmon increased by 27% compared to the Ecosim baseline.

DISCUSSION

Affected organisms

The organisms that could be affected by the presence of large escapes of GH coho in the SoG ecosystem are more than just their prey, predators and conspecifics, and could include multiple trophic levels. However, effects on ecosystem components could only be detected when very extreme introduction scenarios were examined, whereas replacement of normal coho salmon with GH coho, or adding an equivalent biomass of the GH coho to the ecosystem, had non-significant effects, indicating that trophic effects required high biomass of introduced fish to disturb the resilience of the modelled ecosystem. Trophic interaction in aquatic systems can be quite simple or complex. For example, Nile perch have predated many native fish to extirpation in Lake Victoria (see Pitcher & Hart 1995). A cascade occurred in California Bay, where some worms and crustaceans increased in response to the presence of invasive green crab that significantly decreased their prey population (Grosholz *et al.* 2000). Our study reports complicated indirect trophic interactions caused by the presence of GH coho, arising from sharing of predators and prey forage fish and competitors, requiring careful quantitative estimations. Although ecological studies on escaped non-transgenic Atlantic salmon have focused only on the effects on the wild conspecific population (Hutchings & Fraser 2008; Liu *et al.* 2013), our model shows that the whole ecosystem could be differently affected throughout the food web by the introduction of a large number of GH coho.

Factors influencing the effects of GH fish

Our model reveals that the effects of GH coho depend on their biomass, diet, environment conditions, and structure of the invaded ecosystem. Our study is consistent with most invasion impact frameworks that identified abundance (or biomass) of invader as a key impact factor: the greater the abundance of the invader, the higher the impact (Parker *et al.* 1999; Ricciardi 2003; Thomsen *et al.* 2011). The effects would become stronger if large numbers of either GH coho or wild-type coho salmon were introduced. Although many frameworks do not include metrics quantifying how abiotic conditions or available resources modify effects of introduced species (Thomsen *et al.* 2011), our study also shows effects vary depending on diets used by GH coho, indicating a strong need for accurate knowledge regarding the natural diets of organisms in the food web. For example, resident salmon were most affected during strongest competition when GH coho fed on the same diet, and least affected during weak competition, such as the forage diet of GH coho. Our model also demonstrates that climate-driven changes in lower trophic levels, such as in biomasses of euphausiids and herring, can greatly alter the whole ecosystem, as observed in other ecosystems (Smith *et al.* 2011; Kaplan *et al.* 2013). Our modelling indicates that climate-driven bottom-up effects can be stronger than the introduced species and thus complicate predictions of the effects of invasive fish under altered ocean conditions.

Our results highlight the role of the structure and key trophic linkages of an invaded ecosystem in shaping the effects of the introduced species. The harbour seal-hake-herring trophic triangle is a clear example where Li *et al.* (2010) showed that removing seals would threaten herring due to increased predation by hake. Except when harbour seals increased by less than 10% (see the forage diet in Fig. 2*b*), all scenarios explored in the current study agree with Li *et al.* (2010) in that harbour seals show an inverse relationship with hake and the same trend as herring. The present modelling suggests that introduced GH coho are unlikely to alter this trophic triangle relationship greatly, in spite of many direct or indirect trophic interactions with each component of the triangle. This also explains why the results of decreased euphausiids (equivalent to decreased hake because euphausiids are the major prey of hake) are similar to those of increased herring in the climate change scenarios. We suggest that ecosystems may have different responses to the introduced species depending on the key trophic linkages of the invaded ecosystems.

Minor trophic linkages may also play an important role in ecosystem functioning. It is important to include minor diet items in ecosystem models because most models cannot create new trophic pathways (Ainsworth *et al.* 2010). Previous studies have shown that overlooking minor diet items in the diet composition of predators can result in incorrect assessments of predation effects on minor prey species (Walters & Kitchell 2001; Christensen & Walters 2004). In our study, resident salmon and GH coho are minor diet items for harbour seal. However, with greatly increased biomass of the minor prey, predatory harbour seal populations were found to substantially increase. Therefore, including the minor diet items in the model is also important to investigate the effects of minor prey on the predators.

Model uncertainty

Our sensitivity analyses reveal that Ecosim predictions are robust to uncertainty in GH coho parameters in both Ecopath and Ecosim. All the trends (increases or decreases) in all functional groups were the same as in the Ecosim baseline that forced elevated GH coho or declining euphausiid biomass. EwE involves numerous parameters, and model uncertainty has long been an issue of consideration (Christensen & Walters 2004). A Monte Carlo simulation routine has been used to improve Ecopath inputs to fit Ecosim to historic time series data (Hoover *et al.* 2013), and different vulnerabilities have been applied to examine how the Ecosim predictions change (Harvey & Kareiva 2005; Li *et al.* 2010). Model uncertainty in our study applied both Monte Carlo and Ecosim sensitivity analyses in an effort to compare sensitivity of Ecopath and Ecosim parameters.

Our study also showed that Ecosim predictions were more sensitive to vulnerabilities than to Ecopath parameters such as production rate and consumption rate. The top-down control by GH coho in the predator-prey interactions amplified the trends in Ecosim predictions. By contrast, consumption rate perturbations did not make a large difference, despite the wide range of possible consumption rates that were considered. We found that GH coho consumption rate remained at only four in the ecosystem simulation of Ecosim baseline (14.6 in the Ecopath model baseline) due to a density-dependent mechanism that occurs when the biomass of GH coho was forced to be at high levels. This suggested that GH coho were restricted by food availability and could not achieve their full growth potential. However, vulnerability can determine predation level and may be the most sensitive parameter in EwE models (Christensen & Walters 2004). Strong top-down control by setting a high prey vulnerability to GH coho, elevated GH coho actual consumption rate to 11 (although they still are undernourished to some extent compared to the value of 14.6 in the Ecopath baseline) when the biomass of GH coho was forced to constantly remain 10 times its baseline. This indicated that impacts of GH coho were indeed larger (Fig. 3*b*) when their actual consumption rate was higher. The strong response to vulnerability parameter values suggested that future studies should include detailed sensitivity analyses for vulnerabilities of prey to their predators.

Although we have considered a wide range of uncertainty in GH coho parameters, we recognize that variability in parameters of other groups (such as biomass of resident salmon) and many other physiological and ecological factors would play important roles in model predictions. For example, we assume GH coho predators are the same as those of resident salmon, which may not be the case. Further, the actual parameter values for GH coho used in the present study have been generated from only a small set of empirical studies conducted in laboratories. The phenotype of GH coho is extremely plastic to environmental conditions, and further the responses of these animals and wild-type coho salmon distinctly to those different conditions. Such genotype-environment interactions complicate predicting the actual parameter values in nature (Bessey *et al.* 2004; Devlin *et al.* 2004*b*, 2006; Sundström *et al.* 2007; Lõhmus *et al.* 2010), and hence also parameter values that should be used in ecosystem modelling exercises.

The present study simulates the trophic effects of an introduced genetically engineered organism into an ecosystem, prior to that organism ever being in nature. Lacking invasion history data is likely to be a major challenge when predicting ecological impacts of introduced species (Ricciardi 2003), and even for cases with data from historic invasions, it is hard to detect indirect interactions and effects (Falk-Petersen *et al.* 2011). Our study has not included the scenario where GH coho salmon could breed in nature (we assumed sterile strains were introduced), but clearly effects could be considerably different if changes in numbers of these organisms were being altered by reproduction. Similarly, we have not considered effects of differential dispersal, migration, and longevity compared to wild type, or effects of modification of the phenotype of the transgenic salmon arising from selection and adaptation. Ideally, fusing fitness-based models (for example Muir & Howard 2002; Ahrens & Devlin 2011) with ecosystem models, as performed here, will assist in providing more refined predictions of potential ecosystem impacts.

ACKNOWLEDGEMENTS

We thank Jim Irvine, William Cheung, Yajie Liu, Robert Ahrens, Alison MacDiarmid and Murdoch McAllister for useful discussions on the interactions between GH transgenic coho salmon and the wild population. We are grateful to Villy Christensen and Cameron Ainsworth for insightful discussions on the Monte Carlo approach, and Jeroen Steenbeek and Joe Buszowski for technical assistance in improving the Monte Carlo function in EwE 6. We also very much appreciate suggestions from the Editor and Associate Editor, and an anonymous reviewer, which greatly improved this manuscript. This work was part funded by the Canadian Regulatory System for Biotechnology to RHD and a BRITE Graduate scholarship to the first author by the UBC Biodiversity Research Centre.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0376892914000319

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