DFO Comments on: DRAFT STUDY ON RISK ASSESSMENT: APPLICATION OF ANNEX I OF DECISION CP 9/13 TO LIVING MODIFIED FISH.

The report gathers information on living modified organisms that are fish and associated risk assessment guidance. In general, this is accomplished, however there are also a number of inaccuracies in the document that are captured in the comments below and need to be addressed. Also, too much time spent on AAS and detailing the intricacies of the molecular genetics behind this one example. The document would benefit from taking a more generic look at growth enhancement as a trait that has been altered in many species of fish.

Throughout: make sure any similar references (e.g. Devlin et al. 2004a and 2004b) are cited with distinguishing letter next to year.

Page 7 Ln 42: suggested change “that informs accurate predictions”

Page 9 Ln 18: add reference “(see also: Devlin et al., 1994; Leggatt et al., 2012)”

Reference: Devlin RH, Yesaki TY, Biagi CA, Donaldson EM, Swanson P, Chan W-K (1994) Extraordinary salmon growth. Nature 371:209-210. doi:10.1038/371209a0.

Page 9 Ln 22: remove “albino rainbow shark….Leggatt, 2019)” as this line incorrectly implies the LM shark line was created by Leggatt. A more relevant reference would be the following as it was the basis for creation of the GloFish company producing all LM tropical fish in USA and Canada “, several fluorescent colours of zebrafish (*Danio rerio*, Gong et al., 2003),”.

Reference: Gong Z, Wan H, Tay TL, Wang H, Chen M, Yan T (2003) Development of transgenic fish for ornamental and bioreactor by strong expression of fluorescent proteins in the skeletal muscle. Biochem Biophys Res Commun 308:58-63. 10.1016/s0006-291x(03)01282-8

Page 10 Ln 13-14: “Transgenic ornamental zebrafish (*Danio rerio*) and barb fish are now being marketed in the United States of America (USA) and Canada.” Would be more accurately written as “Transgenic ornamental zebrafish, tetras (*Gymnocorymbus ternetzi*), barbs (*Puntigrus tetrazona*) and rainbow shark (*Epalzeorhynchos frenatum*) are now being marketed in the United States of America (USA), and zebrafish and tetras in Canada.”

Page 10 Table 1: At the bottom of column 1, ‘ornamental’ is not a trait, it’s a type of fish. Instead could use ‘fluorescence’ or ‘colour’. There would be several species such as golden danio, tiger barb, white tetra, so on.

Page 11 Ln18: “They have been placed on the market in Canada and USA.” While they are technically approved in USA, they have yet to enter the food market (although likely will this year or next)

Page 11 Ln35: delete “sockeye and” as reference cited discusses Coho salmon only (and reference should be listed as 2004a)

Page 11 Ln41: remove paragraph mark

Page 11 Ln44-46: Should reference Du et al. 1992 here.

Page 11 Ln46-48: Reference cited here (Devlin et al., 1995 - a or b?) is not appropriate as Devlin et al. did not specifically examine the expression of GH via ocean pout promoter, rather reported on the resulting accelerated growth.

Page 11 Ln 51, Paragraph continues onto page 12 Ln 1 - 8. There is a lot of detail here regarding the molecular characterization of the AquAdvantage salmon (AAS). There is a lot more detail here than for other transgenic traits. Consider removing paragraph or making entire section more generic. The information is very detailed and looks out of place here. A lot of the information is repeated in section 6.2.

Page 12 Ln 1-2: This sentence gives impression a part of the vector has been incorporated along with the construct, which is not the case. Other sections on fatty acid composition, enhanced double muscle, disease resistance and so on, don’t give these types of details.

Page 12 Ln 7: Are there two genes, or one gene and a promoter? Too much detail for this organism relative to the other organisms.

Page 13 Ln49-51: more accurately written as “For example, Noble et al. (2017) developed LM zebrafish expressing thiaminase, that would be theoretically dependent on thiamine in their diet and consequently die if they escaped, although the models developed were not fully rescuable by dietary thiamine.” Or “For example, Noble et al. (2017) explored the use of a thiaminase transgene in LM zebrafish, that would result in fish being depended on thiamine in their diet and consequently die if they escaped”

Page 14 Section 5.3.7: This section should include a paragraph on the GloFish.

Page 14 Ln20: change to read “GFP and other fluorescent protein transgene reporter systems”, and change “GFP” to read “fluorescent protein transgenes” when GFP is being used in general terms in this section. Many different fluorescent protein transgenes are used as reporting genes in zebrafish research, often several different transgenes in the same model to tag different genes.

Page 14 Ln27: add to end of paragraph “and many research models now utilize multiple fluorescent colours in a single LMF (e.g. Pan et al., 2013)”

Reference: Y. Albert Pan, Tom Freundlich, Tamily A. Weissman, David Schoppik, X. Cindy Wang, Steve Zimmerman, Brian Ciruna, Joshua R. Sanes, Jeff W. Lichtman, Alexander F. Schier. 2013. Zebrabow: multispectral cell labeling for cell tracing and lineage analysis in zebrafish. Development 140: 2835-2846; doi: 10.1242/dev.094631

Page 14 Ln29-30: was it medaka or zebrafish that was the first marketed fluorescent fish? I think zebrafish, and this is what is stated Page 15 Ln 38.

Page 14 Ln39-40: remove scientific names or change to “tetrazona”.

Page 14 Ln40: change “They” to “Fluorescent LM tropical fish”

Page 15 Ln1-4: Reference for this sentence?

Page 15 Ln 32: Spelling, AquAdvantage

Page 15 Ln 38-41. A few inaccuracies with how this is written. Tetra’s, tiger barbs and sharks are GloFish products by Yorktown, USA so should be in the following paragraph. TK-1-3 are the medaka and zebrafish models produced originally by Taikong (http://www.azoo.com.tw/, different from the zebrafish produced by GloFish mentioned in the following paragraph). The cichlids and angelfish I think were produced by academic/government agencies not Taikong Corp, and I haven’t been able to confirm that they are for sale in Taiwan (if you have information that they are for sale, please ignore)

Page 15 Ln43: Should add more information here regarding the commercialization and risk assessment of GloFish.

Page 16 Ln15-21: This paragraph has little do with fluorescent fish. Consider removing or moving.

Page 17 Ln5: “which are sterile (Devlin et al., 2010).” Would be more accurately written as “which are sterile and the technique is generally >98% effective (Devlin et al., 2000)”.

Page 17 Ln6-9: Inaccurate as written. Better option would be “In addition, AquaBounty’s triploid commercial form is also all-female, preventing reproduction of any diploid exceptions in the population in areas without Atlantic salmon”. As for the “enhanced growth rate due to their polyploidy”, triploid fish tend to grow slower than diploid so this part is inaccurate and I recommend deleting. Triploid all-female populations do remove the possibility of premature maturation in Atlantic salmon which is costly for the industry, so maybe this is what you were referring to?

Page 17 Ln19-20: Consider changing to:”…freshwater research facility on Prince Edward Island, Canada, where eggs and fish cannot escape.”

Page 17 Ln46: Consider changing to: “In addition, facilities conducting research on AAS are operating in…”

Page 18 Ln21-27. Recommended alternate wording “Environment and Climate Change Canada (ECCC) administers LMF applications under the Canadian Environmental Protection Act, 1999 (CEPA, 1999), and the New Substances Notification Regulations (Organisms) (NSNR[O]). Any request to develop fish using modern biotechnology for commercial purposes is subject to the NSNR[O] under CEPA, 1999. Fisheries and Oceans Canada (DFO) is the primary authority on fish in Canada, and works with ECCC and Health Canada (HC) to provide science advice in the form of scientific risk assessments to ECCC for any LMF notified under CEPA. Health Canada is the agency responsible for determining if LMF notified for human consumption are safe as food”

Page 21 Ln18. Regarding “certain types of aquaria”. While aquaria in general are well contained, use of LMF in home aquaria has high probability of fish release due to the well-documented habit of owners releasing unwanted pet fish to natural water systems.

Page 21 Ln 29: add to the end of the sentence “, although data conflicting these results have been reported (e.g. Cortemeglia & Beitinger 2006b, Jha 2010, Gong et al., 2003).” NOTE: delete Cortemeglia & Beitinger (2006b) reference earlier in this sentence as they found no effect of fluorescence on predation.

References: Jha, P. 2010. Comparative study of aggressive behaviour in transgenic and wildtype zebrafish Danio rerio (Hamilton) and the flying barb Esomus danricus (Hamilton), and their susceptibility to predation by the snakehead Channa striatus (Bloch). Ital. J. Zool. 77(1): 102-109.

Gong, Z., Wan, H., Tay, T.L., Wang, H., Chen, M., and Yan, T. 2003. Development of transgenic fish for ornamental and bioreactor by strong expression of fluroescent proteins in the skeletal muscle. Biochem. Biophys. Res. Commun. 308: 58-63.

Page 21 Ln35: Suggest removing ‘predation-enhancing fluorescence’.

Page 21 after Ln52: If appropriate to mention here “DFO has conducted scientific environmental risk assessments of notified fluorescent zebrafish and tetras and concluded there was low environmental risk of these fish under commercial use in Canada due to their expected inability to overwinter in Canadian waters (e.g DFO 2019).

Reference: DFO. 2019. Environmental and Indirect Human Health Risk Assessment of the GloFish® Tetras (*Gymnocorymbus ternetzi*): Five Lines of Transgenic Ornamental Fish. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2019/002.

Page 22 Ln 7-11: Reports on effects of fluorescent protein transgenesis on predation in the scientific literature are conflicting (see above) and no study directly compares equally-reared, related, non-transgenic and transgenic fish. Consequently the effects on predation are sill unclear so I would delete reference to assuming they’ll be preyed on at a higher rate, or add caveats to that.

Page 22 Ln16-18: Regarding “Release or escape of this fish could provide the opportunity for it to move over a wider range of marine environments and different river systems, in favourable climatic environments.” What is meant by this? How does the fluorescent transgenic state influence the ability of the fish to move over environments?

Page 23 and throughout: When referencing Devlin et al., 2015 it is best to put “see Devlin et al., 2015” unless specifically referencing conclusions from that paper, since this is a review article.

Page 23 Ln12: Oke et al. 2013 did not demonstrate introgression of transgenes into trout populations as the sentence implies. Oke et al. did find the transgene could be transmitted to Atlantic salmon x trout hybrid offspring. Could maybe change the sentence to read “Oke et al. (2013) demonstrated that this fertility can include hybridisation with brown trout, and transmission of the transgene to hybrid offspring”

Page 23 Ln23-24: regarding the sentence: “For example, studies in semi–natural arenas with Coho salmon suggest that cultured GH salmon are reproductively out-competed by wild-reared salmon (Bessey et al., 2004; Fitzpatrick et al., 2010).” It was later shown by Leggatt et al. 2014 that decreased reproductive success was due in large part to culture conditions, and the reproductive success of GH salmon reared in nature (e.g. if escaped at an early age) cannot be assumed to be limited. This info could be place lower in the paragraph when discussing uncertainty.

Reference: Leggatt RA, Hollo T, Vandersteen WE, McFarlane K, Goh B, Prevost J, Devlin RH (2014) Rearing in seawater mesocosms improves the spawning performance of growth hormone transgenic and wild-type coho salmon. PLoS ONE 9:e105377. doi:10.1371/journal.pone.0105377

Page 24 Ln24-27: FYI, risk assessments of AquAdvantage salmon in Canada did consider the potential escape of diploid exceptions in the AAS population as well as potential for fertile broodstock to escape (see DFO 2019. Environmental and Indirect Human Health Risk Assessments for the Manufacture and Grow-out of EO-1α Salmon, including the AquAdvantage® Salmon, at a Land-Based and Contained Facility near Rollo Bay, PEI. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2019/014.)

Page 25 Ln1-8: FYI Canada has also imposed conditions on use of AquAdvantage via the Significant New Activities Notice (see <http://www.gazette.gc.ca/rp-pr/p1/2013/2013-11-23/html/notice-avis-eng.html#d106>), and the most recent notification has additional recommended conditions (see Risk Management Section of above DFO 2019 document)

Page 25 Ln12: Spelling, Grow-out

Page 25 Ln33: Section 6.5.2 should be about Biocontainment, not biocontainment of AAS. Again here there is a great deal of detail specific to AAS where it might be better to discuss biocontainment strategies in more generic terms.

Page 26 Ln1: As above, this section should be about physical containment in general.

Page 27 Ln48: Remove second ‘the’

Section 7.1: Number of respondents from North American countries?

Section 8.1.c and d: It is also important to identify relevant non-LMO species/lines for comparator purposes in the risk assessment. Assessments should consider what type of comparator would be appropriate, possibly on a case-by-case basis - i.e. should a wild strain or commercial strain be used as a non-LMO comparator during risk assessments?