BROAD-SCALE, REPEATABLE RESPONSE PATTERNS OF FISH AND INVERTEBRATES EXPOSED TO PULP AND PAPER MILL EFFLUENT

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Pulp and paper mill effluent effects on aquatic biota are measured over a broad geographic scale by the National Environmental Effects Monitoring (EEM) Program in Canada. We have now assessed EEM data collected from receiving environments across Canada through three cycles of data collection (covering the years 1992-2004). The standardized sampling and reporting methodology, extensive database, and subsequent analyses have provided a fairly robust picture of the effects of these effluents in a variety of habitats. The magnitudes and distribution of effects observed for effluent-exposed benthic invertebrates and fish have, for the most part, shown a high degree of consistency through time over the last two cycles of data collection. Over the last decade, the national average response pattern measured for benthic invertebrate communities has been one typically associated with eutrophication, ranging from mild to more pronounced, partly depending on habitat type. More specifically, invertebrate communities exposed to pulp mill effluent have commonly exhibited increases in abundance, together with some combination of increases, decreases or no change in taxon richness, depending on the degree of eutrophication. Over the same time period, the national average response for fish has been one indicative of nutrient enrichment overlaid by metabolic disruption. That is, exposed fish have consistently shown evidence of increased food availability (fatter, faster growing, larger livers) together with disruption of allocation to reproduction (smaller gonads). Thus, although sublethal toxicity testing demonstrated large improvements in effluent quality during the mid 1990's, measurable effects remain in the field. Efforts are currently underway to better understand and address these remaining effects.

Keywords: nutrient enrichment, reproduction, endocrine disruption, invertebrate community, fish, biomonitoring

Introduction

Under the *Fisheries Act*, the 1992 *Pulp and Paper Effluent Regulations* (PPER) prescribe discharge limits for total suspended solids and biochemical oxygen demand, and require effluent to be non-acutely lethal to fish. These limits reflect what secondary treatment of effluents can achieve and provide a national baseline standard that is intended to protect fish, fish habitat and the use of fisheries resources. At the time the regulations were developed, it was acknowledged that there was uncertainty about the effectiveness of the new limits for protecting the diverse variety of aquatic environments receiving pulp and paper mill effluents in Canada.

In order to assess the adequacy of the effluent regulations for protecting the aquatic environment, Environmental Effects Monitoring (EEM) was included as a requirement in the 1992 PPER. In May 2004, the *Regulations Amending the Pulp and Paper Effluent Regulations* (RAPPER) came into force and further clarified the requirements of the 1992 PPER. Canadian pulp and paper mills that are subject to the RAPPER are required to conduct studies on their receiving environments in order to assess and monitor effects potentially caused by their effluent. These studies include two key components that monitor effluent effects on receiving water biota: 1) a fish population survey to assess fish health and 2) a benthic invertebrate community survey to assess effects on fish habitat. Some mills are further required to conduct studies to assess effects on the usability of fisheries resources, including a study of dioxins and furans in fish tissue and a tainting study. In addition, sublethal toxicity testing and measurements of supporting water and sediment quality variables are conducted to contribute to the program in areas such as interpretation of biological data and assessing effluent quality.

The EEM program is structured into “cycles,” whereby a mill conducts an EEM study every three to six years with both monitoring and interpretation phases. At the beginning of each cycle, each mill is required to develop a site-specific study design in collaboration with Environment Canada regional staff. At the end of each cycle, each mill must submit an interpretative report that summarizes its monitoring results. The EEM program uses a tiered approach to monitoring, with initial studies carried out to characterize and assess the condition of the receiving environment followed by targeted or focused studies to determine the extent and magnitude of effects, where effects are detected and confirmed, or a reduced level of monitoring, where effects are not found. Technical guidance is developed by Environment Canada on all aspects of EEM studies, including design, analyses, and interpretation of data (Environment Canada 2005). Additional information on the EEM program is available at http://www.ec.gc.ca/eem/.

In 2004, most mills completed their third cycle of monitoring and reporting. Cycle 1 (completed in 1996) was used primarily as a baseline to gain better understanding of the variability of the field measurements, to identify problems, and to provide recommendations for future cycles. The second cycle (completed in 2000) results showed that mills were successful in reducing the toxicity of their effluent and that their effluent quality had greatly improved since the promulgation of the 1992 PPER (many Canadian mills upgraded their effluent treatment systems just prior to Cycle 2). Despite these improvements, the national assessment of biological monitoring data for Cycle 2 EEM (Lowell et al. 2003, 2004) showed that pulp and paper mill effluents continued to have some effects on fish and benthic invertebrates in the field. The predominant response patterns seen for fish were a decrease in gonad weight and increases in liver weight, condition and weight at age. These response patterns are believed to be indicative of some form of metabolic disruption or impairment of endocrine functioning in combination with a nutrient enrichment effect. The national average response pattern for the benthic invertebrate community surveys in Cycle 2 was one of mild to moderate eutrophication.

In Cycle 3, 112 mills conducted EEM studies, including 29 marine/estuarine and 66 freshwater field surveys, with the remaining mills focussing on the non-field survey components of the program. There were 62 standard fish field surveys, which included four studies using molluscs, and 87 standard benthic invertebrate field surveys; all mills conducted sublethal toxicity studies in the laboratory. A small number of mills conducted alternative field studies, rather than the standard fish and benthic invertebrate field surveys; these alternatives included caged bivalves, mesocosms, and other research-oriented approaches. In total, 24 mills were exempted from conducting a fish survey because the effluent concentration in the exposure area was less than 1% within 250 m of the point of discharge. Additional site-specific exemptions were given for various reasons, such as unsafe conditions for sampling and lack of suitable reference areas. The Cycle 3 totals for mills conducting the fish and benthic invertebrate field surveys, as well as alternative studies and sublethal toxicity testing, were similar to those for Cycle 2.

Magnitudes and Distribution of Effects Measured in the Fish Survey

The adult fish survey is used to determine if the effluent is affecting fish populations in the field by comparing effluent-exposed fish with those from reference areas. The survey uses fish reproduction, condition, growth, and age structure to assess the overall health of exposed fish. These are assessed via measurements of five core fish endpoints (Table 1; Lowell et al. 2002, Environment Canada 2005). Due to difficulties with aging fish at some sites, our analyses have particularly focussed on gonad weight, liver weight, and relative body weight (but see Lowell et al. 2003, 2004, 2005 for additional analyses of age and growth).

Table 1: Core endpoints for fish survey

Endpoints	**Endpoint provides information on:**
Weight of gonads relative to body weight	Reproduction
Liver weight relative to body weight	Condition
Body weight relative to body length	Condition
Age	Survival
Body weight relative to age	Growth

The range and distribution of effects measured in Cycle 3 were strikingly similar to those measured in Cycle 2 (Fig. 1). Magnitudes of effects (exposure vs. reference percent differences) in Figure 1 were calculated as exposure area minus reference area adjusted means, expressed as a percentage of the reference area adjusted mean. Adjusted means (gonad, liver, or body weight means adjusted for body weight or length;

Table 1) were derived from Analysis of Covariance (ANCOVA). Figure 1 focuses on comparisons where exposure versus reference ANCOVA slopes were parallel (the majority of comparisons; Lowell et al., 2003, 2005).

Similar to Cycle 2, Cycle 3 condition factor showed the narrowest range in exposure versus reference area percent differences (-15% to 25%). Cycle 3 gonad weight percent differences ranged from approximately -90% to 70%, while liver weight differences ranged from -55% to 120%, again with a highly similar data distribution to that observed in Cycle 2. Thus, at a national level, we observed a high reproducibility between Cycles 2 and 3 in the magnitude and distribution of measured effects. This is likely related to the consistency of response patterns from one cycle to the next (Lowell et al., 2005).

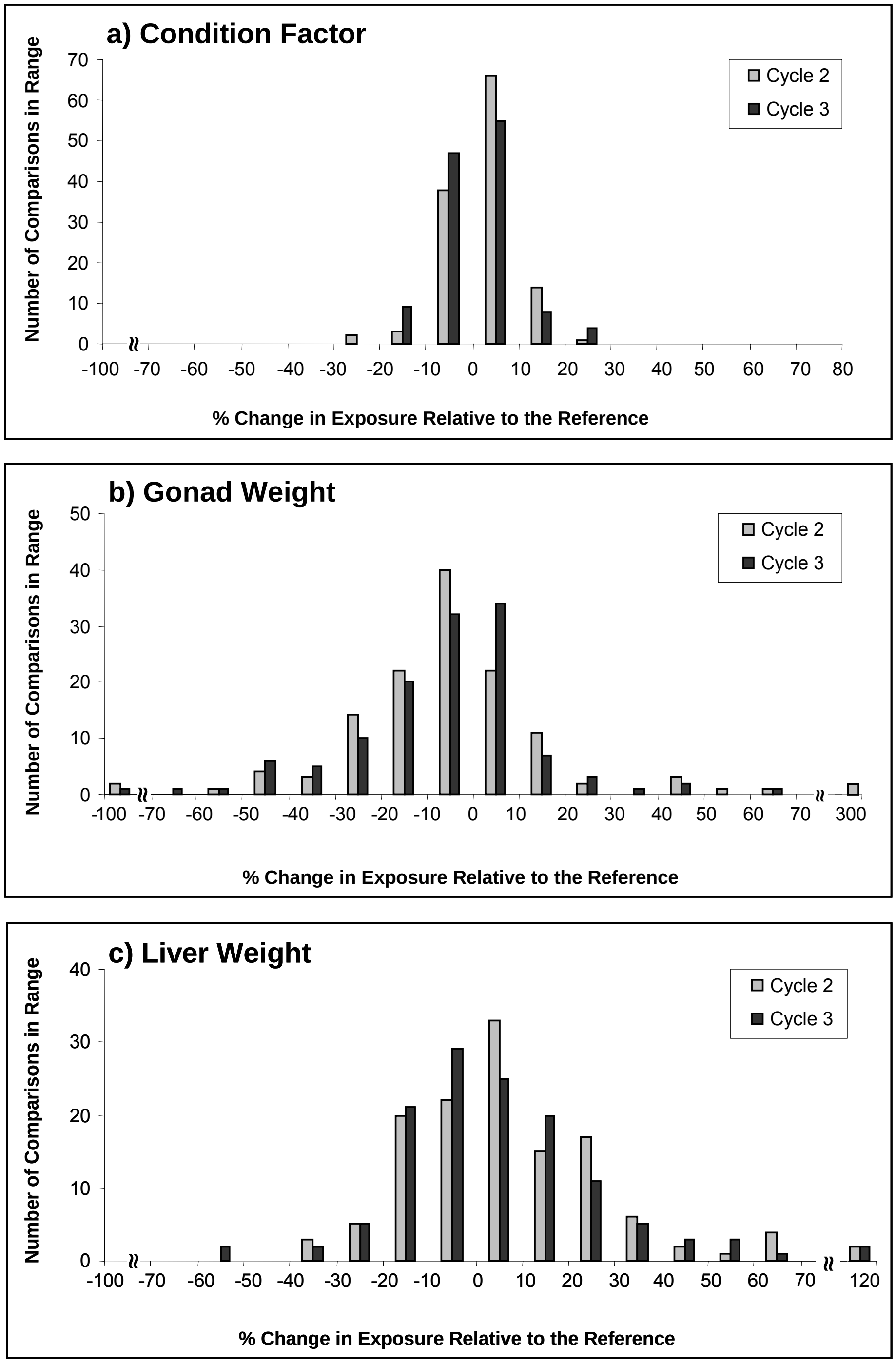

Figure 1: Distribution of measured percent differences between exposure and reference area fish in Cycles 2 and 3 for: a) condition factor, b) gonad weight and c) liver weight.

Magnitudes and Distribution of Effects Measured in the Benthic Invertebrate Community Survey

The second primary component of the EEM program is the benthic invertebrate community survey, which assesses the impacts of mill effluent on fish habitat. The benthic invertebrate survey helps to supply information on the aquatic food resources available for fish and on the degree of habitat degradation due to organic enrichment or other forms of physical and chemical contamination. The four endpoints used to assess the effects of pulp and paper effluent on benthic invertebrate communities in the field are given in Table 2 (Lowell et al. 2002, Environment Canada 2005). Taxa were analyzed at the family level (or above when data were reported only at a higher level); for further discussion of the rationale for the level of taxonomic resolution, see Bowman and Bailey (1997), Bailey et al. (2001), Lenat and Resh (2001) and Culp et al. (2003).

Table 2: Core endpoints for benthic invertebrate community survey

Endpoints	Endpoint provides information on:
Total abundance	Number of animals
Taxon richness	Number of taxa or kinds of animals
Simpson's evenness	Measure of how evenly the animals are distributed among the taxa
Bray-Curtis index	Measure of dissimilarity in community composition among sites

The Cycle 3 range and distribution of measured effects for all four endpoints were very similar to those in Cycle 2 (Fig. 2), as was found for fish. This underscores the reproducibility of measured effects on invertebrates at a national scale in Cycle 3 relative to Cycle 2 and likely reflects the consistency of response patterns from one cycle to the next (Lowell et al. 2005). The measured percent differences were calculated as the exposure area mean minus the reference area mean, expressed as a percentage of the reference area mean. Figure 2 does not include mills that conducted a gradient design, since percent differences (as computed here) cannot be calculated for this type of study design. Note, however, that standardized effect sizes can be calculated for gradient designs for the purposes of meta-analysis, which can then be used to investigate patterns of effects (Lowell et al. 2003), and a similar reproducibility of effects between cycles was also observed for gradient designs (Lowell et al. 2005).

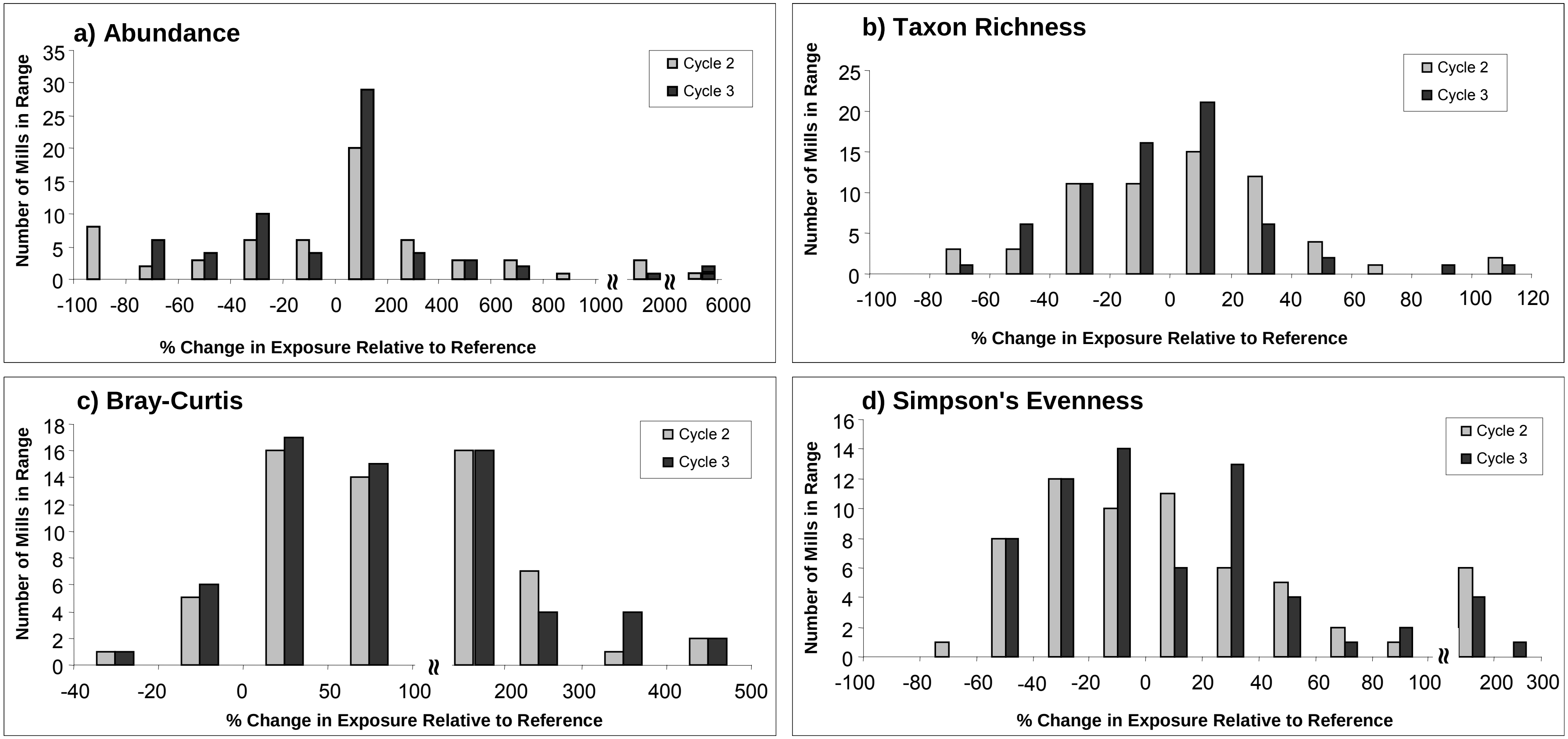

Figure 2: Distribution of measured percent differences between exposure and reference areas for the benthic invertebrate survey (control/impact designs only) for a) abundance, b) taxon richness, c) Bray-Curtis and d) Simpson's evenness.

Similar to Cycle 2, the abundance endpoint showed the most extreme range in Cycle 3, varying from a decrease of 80% to an increase of over 5500%. The percent differences distribution for taxon richness ranged approximately from a decrease of 65% to an increase of 120%. Approximate Cycle 3 ranges for the Bray-Curtis index and the Simpson's evenness index were -30% to 470% and -60% to 230%, respectively – again very similar to those for Cycle 2. Note that most of the Bray-Curtis values were positive, due to the method of calculation. The few negative values were due to unusual data distributions.

Discussion

Having completed its third cycle of data collection and reporting, the EEM program has provided an extensive database and a fairly robust picture of the nature of the effects of pulp and paper mill effluents on receiving water biota across the country. This kind of broad view of effects was not previously available at this geographic scale.

The magnitudes and distribution of effects observed for effluent-exposed fish and benthic invertebrates during Cycle 3 were remarkably similar to those measured during Cycle 2. The repeatability of measured effects between cycles was observed despite changes in study areas, sampling design, and sentinel species between cycles, further underscoring the underlying consistency in effluent effects. These findings are in agreement with meta-analyses that were also conducted on the Cycle 2 and 3 data (Lowell et al. 2003, 2004, 2005). The meta-analyses showed that the national average response pattern measured for fish in both Cycles 2 and 3 was consistent with one of nutrient enrichment overlaid by metabolic disruption. That is, exposed fish have consistently shown evidence of increased food availability or increased food absorption (fatter, faster growing, with larger livers) together with disruption of allocation of resources to reproduction (smaller gonads). This latter metabolic disruption of gonadal development (Munkittrick et al. 1991) may include some aspect of endocrine disruption associated with problems in producing sufficient sex steroid hormones (Van der Kraak et al. 1992, Damstra et al. 2002). Of the approximately 60 mills conducting fish surveys in Cycles 2 and 3, approximately 65 to 70% measured statistically significant changes (most often decreases) in gonad size. Other observed response patterns for fish have included nutrient enrichment without measurable metabolic disruption, as well as patterns typically associated with nutrient limitation or chemical toxicity. These findings agree with a number of research studies that have been carried out at mills across the country (reviewed by Munkittrick et al. 1991, 1994, 2000, 2002, Lowell et al. 2003, 2004).

The national average response for benthic invertebrate communities in both Cycles 2 and 3 was consistent with one of eutrophication, ranging from mild to more pronounced, partly depending on habitat type (Lowell et al., 2003, 2004, 2005). More specifically, benthic invertebrate communities exposed to pulp mill effluent have commonly exhibited increases in abundance, together with some combination of increases, decreases or no change in taxon richness, depending on the degree of eutrophication. Other observed benthic invertebrate response patterns have included those typically associated with toxicity or smothering effects. These response patterns and the mechanisms leading to them have been confirmed by a number of experimental and monitoring research studies conducted at mills across Canada (reviewed by Lowell et al. 1995, 2000, 2003, Chambers et al. 2000; Culp et al. 2000, Lowell and Culp 2002).

It should be noted, however, that some possible small shifts in the patterns of effects measured in the fish and benthic invertebrate field surveys between Cycles 2 and 3 were also observed (Lowell et al. 2005). The fish data have suggested some overall lessening of nutrient enrichment effects (but no change in reproductive effects since Cycle 2). On a habitat-specific basis, the benthic invertebrate data have shown evidence of a possible lessening of toxicity/smothering effects in marine-type habitats, as well as more pronounced eutrophication in freshwater depositional habitats. These shifts for fish and invertebrates may be due to a variety of possible causes. For example, the average reduction in number of invertebrate taxa observed in Cycle 3 in freshwater depositional habitats (sometimes a sign of more pronounced eutrophication) may have led to a reduction in food availability for sentinel fish at some mills (resulting in reduced nutrient enrichment in fish), if the taxa that were lost were also taxa that the fish would normally use as a food source. Alternatively, the shifts in fish and invertebrate responses may have been influenced by changes between cycles in study designs and selection of study areas and sentinel species. It is also possible that the fish and benthic invertebrate response patterns will continue to show slight shifts from cycle to cycle due to natural variability in the receiving environment or to anthropogenic changes through time that are unrelated to exposure to pulp and paper mill effluent. Under this extrinsic variability scenario, these shifts may include either increases or decreases in individual endpoints, sometimes changing direction from cycle to cycle. In this situation, the underlying, time-averaged response pattern may remain approximately the same, unless these extrinsic factors show a long-term, unidirectional trend. Again, the possible shifts in response patterns observed to date are small relative to the overall repeatability of effects over the last two cycles of data collection.

Sublethal toxicity data collected as part of the EEM program have demonstrated pronounced improvements in effluent quality from Cycle 1 to Cycle 2, with, for the most part, no further changes in effluent quality in Cycle 3 (Scroggins et al. 2002, Lowell et al. 2003). It should be noted, however, that the sublethal toxicity reporting methodology and selection of tests do not track all aspects of effluent quality. In particular, they do not currently measure nutrient enrichment effects of the effluent (the most commonly observed effect in the field surveys), although the tests could be modified to provide some information on nutrient enrichment effects. Also of note, and of relevance to the reductions in gonad size measured in the fish field survey, the most sensitive sublethal toxicity tests were those that measured a reproductive endpoint (although these did not include fish tests).

Given that the sublethal toxicity tests do not measure all aspects of effluent quality (particularly nutrient enrichment), it is possible that changes in effluent quality may have contributed to the small shifts in field response patterns outlined above. It should also be noted that some of these shifts may have resulted from gradual recovery from (or, in some cases, added accumulation of) historical effluent deposits in the receiving environment. Further, some of the shifts may have resulted from lag effects. That is, recovery from (or worsening of) some effluent effects may not be evident until some time after release of the effluent causing the effects, as effects are gradually transmitted through complex trophic food webs. Additional information is required to fully address these issues.

The results obtained in future cycles of the EEM program, together with more focused studies at some mills, will help to answer these questions. For example, a series of targeted investigation-of-cause studies are currently being undertaken at key mills to better understand and address the gonad reduction effects that continue to be observed, despite the marked improvements in effluent quality that have been attained since the early 1990's.

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