

1 **A century of limnological evolution and interactive threats in the**

2 **Panama Canal: long-term assessments from a shallow basin**

3 **Running title: Human alteration of tropical rivers**

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20 **Article type: Primary Research Articles**

21 **Highlights**

- 22 • Multi-proxy records reveal the effects of impoundment across a shallow  
23 neotropical reservoir basin in the Panama Canal.
- 24 • Records show that limnological conditions shifted from a swamp-type  
25 environment to a lake system.
- 26 • Despite major human impacts, fluvial hydrology still governs the lake basin  
27 ecosystem functioning.
- 28 • Management of the basin must focus on maintaining long-term river  
29 hydrodynamics.

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31 **Abstract**

32 Large tropical river **prairie dam** projects are expected to accelerate over the forthcoming  
33 decades to satisfy growing demand for energy, irrigation and flood control. When tropical  
34 rivers are dammed, the immediate impacts are well studied, but the long-term (decades-  
35 centuries) consequences of impoundment remain poorly known. We combined historical  
36 records of water quality, river flow and climate with a multi-proxy (macrofossils,  
37 diatoms, biomarkers and trace elements) palaeoecological approach to reconstruct the  
38 limnological evolution of a shallow basin in Gatun Lake (Panama Canal, Panama) and  
39 assess the effects of multiple linked factors (river damming, forest flooding,  
40 deforestation, invasive species, pollution and hydro-climate) on the study area. Results  
41 show that a century of dam construction, species invasion, deforestation and salt  
42 intrusions have forced a gradual change in the study basin from a wetland-type  
43 environment towards a more saline lake-governed system of benthic–littoral production  
44 likely associated with the development of macrophytes. Hydrology still remains the most  
45 important long-term (decades) structural factor stimulating salinity intrusions, primary  
46 productivity growth, deposition of minerals, and reduction of water transparency during  
47 wet periods. During dry periods, physical-chemical conditions are in turn linked to clear  
48 water and aerobic conditions whilst nutrients transform into available forms in the  
49 detrital-rich reductive sediments. Our study suggests that to preserve the natural riverine  
50 system functioning of this area of the Panama Canal, management activities must address  
51 long-term ecosystem structural drivers such as river flow, runoff patterns and physical-  
52 chemical conditions.

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53 **Keywords:** hydrology, tropical rivers, species invasion, palaeoecology, Panama Canal,  
54 river damming.

55 **INTRODUCTION**

56 Many large tropical rivers have been dammed for water management, commerce and  
57 energy production (Grill et al., 2019) and projections show the number of large dammed  
58 (> 15 m high) tropical river projects will increase three-fold over the forthcoming decades  
59 (Gleick, 2003; Zarfl et al., 2015; Poff and Schmidt, 2016). When rivers are dammed, the  
60 immediate impacts are well studied (Poff and Schmidt, 2016): the alteration to the carbon  
61 cycling (Tranvik et al., 2009; Maavara et al., 2017), river networks and flood plains,  
62 mainly through the creation of artificial reservoirs, modification of natural patterns of  
63 sediment transport, restriction of upstream-downstream movement of fish, promotion of  
64 the invasion of alien species that bring about changes in water quality and productivity  
65 (Poff and Schmidt, 2016; Agostinho et al., 1999). Yet almost nothing is known about the  
66 impacts on limnology or aquatic ecology in the long-term (decades-centuries).

67 On decadal time scales, dammed reservoirs may stabilise hydrological conditions,  
68 reduce concentrations of major river-fed geochemical elements (e.g. Mg, Fe) and increase  
69 nutrients, which can promote a gradual homogenization of diatom communities and an  
70 expansion of macrophyte cover and associated invertebrates (Zeng et al. 2018; Wengrat  
71 et al., 2018; Wengrat et al., 2019). However, reservoirs tend to experience unique  
72 environmental conditions and ecological processes. As such, there is no single ecological  
73 baseline for tropical reservoirs against which the extent of degradation and the effects of  
74 multiple stressors can be assessed (Wengrat et al., 2019). This limits our ability to  
75 understand processes in impounded rivers, especially in the neotropics (the tropical areas  
76 of North, Central and South America), where they remain poorly studied (Agostinho et

77 al., 1999; Carvajal-Quintero et al., 2017) with almost no long-term continuous time-  
78 series of data for multiple ecological and environmental variables after dam construction.  
79 Even if continuous data are available (e.g. Wengerat et al., 2019), most records are no  
80 older than a quarter of a century (Agostinho et al., 1999; Wengerat et al., 2019), which  
81 severely limits our understanding of how these aquatic systems can be sustainably  
82 managed in the long-term (decades-centuries).

83 The formation of Gatun Lake (Panama Canal, Central America) through the  
84 damming of the Chagres River in 1913 offers an unrivalled opportunity to explore the  
85 impacts of impoundment over a period of more than 100 years. During this time the lake  
86 system has experienced a suite of changes due to the prospection and construction of the  
87 canal, land use changes, the invasion of exotic species and natural climatic fluctuations.  
88 Extensive environmental and ecological records of these events, unavailable for other old  
89 neotropical dams, are available thanks to monitoring and research programs established  
90 by the Panama Canal Authority (ACP for its acronym in Spanish) and the longstanding  
91 presence of the Smithsonian Tropical Research Institute (STRI) in Panama.  
92 Notwithstanding, these instrumental records are limited to post-damming times, and thus  
93 environmental and ecological changes from pre-dammed times to the present day are still  
94 poorly resolved. The use of palaeoecological techniques can provide continuous data on  
95 sedimentological changes and species abundances over time to help define tropical  
96 limnological histories (e.g. Wengerat et al., 2019, Zeng et al., 2018) and are ideally suited  
97 to application to the Gatun Lake.

98 In this study, we build a biological and environmental chronological sequence of  
99 change in a shallow (< 8 m depth) basin of Gatun Lake from pre-damming times to the

100 present day. We combine historical water quality records (secchi depths, pH, conductivity  
101 and dissolved oxygen) and river annual flow and climate (precipitation) spanning 1972 to  
102 2013 with sediment core record covering around 150 years. The aims of the study are to:  
103 (1) reconstruct the limnological evolution of the study area from pre-impoundment times;  
104 and (2) assess the combined long-term influence of macrophyte development (including  
105 the expansion of invasive species), physical-chemical and hydro-climate variation on  
106 limnological and ecological properties. We focused our study on a shallow basin of the  
107 lake because shallow areas are less influenced by the dredging activities of the Canal, and  
108 have been reported to be more sensitive to the threats of impoundment, such as  
109 macrophyte expansion and species invasions compared to deeper areas of the Canal Zone  
110 (Zaret and Paine, 1973; Von Chong 1986).

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111

## 112 MATERIALS AND METHODS

### 113 Study Site

114 Gatun Lake is situated in the valley of the Chagres River, Panama (9°11'N 79° 53'W)  
115 (Fig. 1). It has a maximum water depth of 30 m in the main basin but extensive shallow  
116 waters (<10 m) in most areas. The lake level is 26 m above sea level storing 5.2 km<sup>3</sup> of  
117 water. When built, Gatun Lake was the first neotropical large prairie dam and the largest  
118 man-made lake in the world with a surface area of 425 km<sup>2</sup>. It serves a dual purpose; as a  
119 channel facilitating global trade and cross-oceanic travel and as a freshwater reservoir  
120 (Gatun Lake) supplying water and hydropower to Panamá City and other towns (Condit  
121 et al., 2001). Around 90% of the total daily freshwater runoff (~ 1.059915<sup>7</sup> m<sup>3</sup>) is

122 discharged through the locks in the operation of the Panama Canal and hydropower  
123 production (Loewenberg, 1999). The lake region experiences a seasonal tropical  
124 monsoonal climate with a mean annual water temperature around 26 °C (Windsor, Rand,  
125 & Rand, 1995). Mean annual precipitation ranges from 1750 mm year<sup>-1</sup> on the Pacific  
126 coast, to 4000 mm year<sup>-1</sup> on the Caribbean coast of Panama (Engelbrecht et al., 2007).  
127 Historical monitoring by STRI at the canal show that annual rainfall has varied quite  
128 substantially between years, apparently related to ENSO conditions (Albrecht et al.,  
129 2017).

### 130 **Environmental and ecological history**

131 The Gatun dam flooded 480 km<sup>2</sup> of rainforest (Heckadon Moreno 1993; McCullough,  
132 2001), and led to an increase in sediment accumulation (ACP, 2012; Loewenberg, 1999).  
133 Post-canal deforestation increased in the watershed area (Wadsworth, 1978; Condit et al.,  
134 2001) and exotic aquatic species were introduced (Von Chong, 1986, Zaret and Paine,  
135 1973). The Asian macrophyte *Hydrilla verticillata* for instance, was first recorded in the  
136 lake around the 1930s (Von Chong, 1986), which, along with two resident water hyacinth  
137 species (*Eichhornia azurea* and *Eichhornia crassipes*), rapidly dominated the lake after  
138 it was filled (Von Chong, 1986). Introduction of the South American peacock bass  
139 (*Cichla ocellaris*) to the lake in 1967 caused a major ecological reorganization associated  
140 with dramatic declines in native littoral planktivorous fish species (Zaret and Paine,  
141 1973), the impacts of which endure 45 years later (Sharpe et al., 2017; Geladi et al.  
142 2019). Other introductions include the Asian bryozoan *Asajirella gelatinosa* (Wood and  
143 Okamura, 1998), the red-rimmed melania snail *Melanooides (Thiara) tuberculata* (Garcés

**Commented [RN7]:** suggests it was deliberate. Is that right?

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144 and Garcia, 2004) and the South American apple snail *Pomacea bridgesii* (Angehr,  
145 1999). Many other taxa are likely to have been introduced but have yet to be documented,  
146 and future introductions will probably continue or increase consistent with traffic through  
147 the Panama Canal (Muirhead et al., 2015).

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148 Salinity in Gatun lake has been historically below 0.2 ppt (Jongeling et al., 2008) but  
149 some areas near the Miraflores locks on the Pacific side (Fig. 1), have exceeded United  
150 States Environmental Protection Agency (EPA) drinking water standards (>3.0 ppt)  
151 during the dry season (Jongeling, et al., 2008). The 2004-2015 expansion of the Panama  
152 Canal appears to be increasing salinity further by (1) increasing demand for water and (2)  
153 using a “water saving basin design” which allows water to migrate vertically (Wijsman,  
154 2013). Observations of non-native brackish water fauna, such as the North American  
155 Harris mud crab (*Rhithropanopeus harrisi*) (Roche et al., 2009) and the Iraqi crab  
156 (*Elaménopsis kempfi*) (Kam et al., 2009), and increases in observations of marine fish in  
157 the lake (Sharpe et al., 2017) all imply increasing salinity. We recommend further  
158 research into this question given the major ecological and evolutionary implications for a  
159 removal of the freshwater barrier to interoceanic dispersals of taxa.

#### 160 **Sample site and core extraction**

161 An 87 cm-long sediment core (LGAT1) was retrieved in 2013 from near “La Represa”  
162 village in the southwest area of the lake (9° 2'49.58"N, 79°50'6.33"W; Fig. 1). The  
163 coring site was selected because it lies outside the dredging zone of the canal and is  
164 located within one of the most deforested areas of the lake's watershed. The core was  
165 retrieved from a semi-littoral area with abundant floating and submerged vegetation at a

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166 water depth of 1 m. We used a Livingstone Piston Sampler of 4 cm diameter. The  
167 sediment-water interface of the core was well preserved and sediment samples were  
168 extruded in the field at 1-cm intervals.

#### 169 **Core chronostratigraphy**

170 Dried sediment samples from core LGAT1 were analysed for  $^{210}\text{Pb}$ ,  $^{226}\text{Ra}$ ,  $^{137}\text{Cs}$  and  
171  $^{241}\text{Am}$  by direct gamma assay in the Environmental Radiometric Facility at University  
172 College London, using ORTEC HPGe GWL series well-type coaxial low background  
173 intrinsic germanium detectors.

#### 174 **Palaeo-proxies**

175 The following geochemical and biological proxies were utilised to build as broad a  
176 picture as possible of limnological change over time. Differences in the amount of  
177 sediment sample analysed per proxy varied according to methodology and budget.

#### 178 *Geochemical analysis*

179 Elemental composition on the 1-cm-thick samples was measured via X-Ray Fluorescence  
180 (XRF) on a handheld analyser spectrometer, XMET 7500. Three grams of each sediment  
181 sample was dried, ground and homogenized using a mortar and pestle and covered with a  
182 Chemplex thin-film sample support. The XRF analyser spectrometer was calibrated  
183 against certified material prior to analysis (Davies et al., 2015) and median values for  
184 each element determined from duplicate measurements. Sampling resolution was at 2-cm  
185 intervals for the top 50 cm of the core and at 4-cm for the remainder. A total of 34  
186 samples were analysed for calcium (Ca), potassium (K), iron (Fe), manganese (Mn),  
187 titanium (Ti), lead (Pb), copper (Cu) and zinc (Zn) concentrations. The elements Pb, Cu

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188 and Zn were used as proxies for human-derived pollution events, Ca as a proxy of  
189 salinity, and K as a proxy of river physical erosion (Davies et al., 2015). We calculated  
190 complementary index ratios according to Davies et al. (2015) to investigate changes in  
191 sediment reduction conditions (Fe/Mn) and detrital inputs (Ti/Ca.).

#### 192 *Biomarkers*

193 We analysed *n*-alkane composition in 10 sediment samples. We used *n*-alkanes C<sub>15</sub>–C<sub>31</sub>,  
194 as indicators of terrestrial plants, macrophytes, and bacteria-algae (e.g. Eglinton and  
195 Eglinton, 2008). Compounds were measured with a Shimazu GC-2010 gas  
196 chromatograph interfaced to a Shimazu GCMS-QP2010 (for detailed methodology see  
197 Kim et al., 2017).

198 We also calculated the terrigenous aquatic ratio (TAR), which quantifies the *in situ*  
199 algal vs. terrestrial organic matter (Ficken et al., 2000) and the submerged/floating  
200 aquatic macrophyte inputs vs. emergent/ terrestrial plant input ratio (Pmar-aq). The Pmar-  
201 aq quantifies the non-emergent aquatic macrophyte input to lake sediments relative to  
202 that from the emergent aquatic and terrestrial plants (Ficken et al., 2000). Values of  
203 Pmar-aq <0.1 correspond to terrestrial plants, 0.1-0.5 to emergent macrophytes and >0.5-  
204 1 to submerged/floating macrophytes (Ficken et al., 2000)

205 The methane index (MI) that quantifies the relative contribution of methanotrophic  
206 Euryarchaeota against ammonia oxidizing Thaumarchaeota was also calculated (Zhang et  
207 al., 2011). MI values close to 1.0 indicate anaerobic environments, whereas values close  
208 to zero indicate aerobic conditions (Zhang et al., 2011). To calculate the MI index we  
209 measured glycerol dialkyl glycerol tetraethers (GDGTs) using an Agilent 1260 UHPLC

210 coupled to a 6130 quadruple MSD high performance liquid chromatography-atmospheric  
211 pressure chemical ionization-mass spectrometry (HPLC-APCI-MS). For detailed  
212 methodology see De Jonge et al. (2014).

### 213 *Macrofossils*

214 We analysed 23 sediment samples for plant and invertebrate relative abundance remains.  
215 Between 2-4 g of dried sediment material per sample were used and all samples were  
216 disaggregated in 10% potassium hydroxide (KOH) before sieving. Macrofossils were  
217 retrieved from the residues of sieved core material (using mesh sizes of 355 µm and 125  
218 µm) following standard methods (Birks, 2001). Macrofossil relative abundance data were  
219 standardized as the number of fossils per 100 cm<sup>3</sup> and identified by comparison with  
220 reference material and by using relevant taxonomic keys (e.g. Birks, 2001). Due to poor  
221 preservation of the macrophyte *Hydrilla* remains, we estimated temporal abundances  
222 through its well-documented historical records (Von Chong, 1986; ACP, 2012) and  
223 expressed on a 0-3 scale, where 0 is absent and 3 highly abundant. All macrophyte taxa  
224 were then classed according to preferred growth-type as: submerged; anchored-floating;  
225 free-floating and emergent. Invertebrate taxa were classed according to feeding behaviour  
226 or preferred habitat as: filter-feeding (bryozoans); macrophyte/detritus (chironomids),  
227 shredders (Trichoptera larvae); benthic (chironomids), and grazers (molluscs and  
228 cladocerans).

229 *Diatoms*

230 Twenty-three samples were analysed for diatom relative abundances following standard  
231 procedures by Battarbee et al. (2001). The diatom suspension was mounted on slides with  
232 Naphrax® after the removal of carbonates by HCl and organic matter by H<sub>2</sub>O<sub>2</sub>. Diatom  
233 taxonomy and ecology mainly followed ‘Diatoms of North America’  
234 (<https://diatoms.org>) and Krammer and Lange-Bertalot (1986; 1991a,b; 1997). Diatom  
235 species were then classed by preferred habitat type according to available literature on  
236 subtropical (e.g. Gell and Reid, 2014; Reid et al., 2018) and neotropical (e.g. Velez et al.,  
237 2005; Velez et al., 2013; Fayó et al. 2018) aquatic system studies. These groups were:  
238 tychoplanktonic, planktonic—associated with freshwaters; planktonic—associated with  
239 brackish waters; planktonic—salinity-tolerant; benthic—associated with freshwaters; and  
240 benthic—salinity-tolerant (see Table S1 for details). For each sediment sample, we  
241 counted a minimum of 400 diatom valves.

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## 242 **Environmental archives**

243 Historical data on physical-chemical and hydro-climatic variables are presented in Table  
244 1. Hydro-climatic, i.e. precipitation (three and five years average) and river annual  
245 discharge data, from 1972 to 2013 were obtained from STRI’s Physical Monitoring  
246 Program ([https://biogeodb.stri.si.edu/physical\\_monitoring/](https://biogeodb.stri.si.edu/physical_monitoring/); Steve Paton, pers. comm.)  
247 and the Panama Canal Authority (ACP, 2012). Physical-chemical variables from 1972-  
248 2013 (pH, conductivity, dissolved oxygen [DO], nitrates, phosphorus, chlorophyll *a* [Chl-  
249 *a*] and secchi depth) were obtained from literature (e.g. Zaret, 1984; Gutiérrez et al.,  
250 1995) and from the ACP Water Quality Monitoring Division reports

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251 (<https://micanaldepanama.com/nosotros/cuenca-hidrografica/>). For the ACP data we used  
252 the mean annual values of each selected variable recorded at two sampling stations  
253 (Laguna Alta–LAT and Toma de Agua Represa–TAR) located in the study basin.

## 254 **Data analyses**

### 255 *Selection of variables*

256 We first assessed collinearity between historical physical-chemical variables and between  
257 hydro-climatic variables using Spearman's correlation coefficients and the Variance  
258 Inflation Factor (VIF). The VIF method accounts for non-linear relationships, which may  
259 remain undetected under normal Spearman's correlation analysis (Feld et al., 2016). We  
260 used a VIF threshold of  $< 7$ , following Zuur et al. (2007). The relationship between  
261 excluded parameters and those included in the analyses is addressed later in the  
262 discussion.

### 263 *Limnological Evolution (Objective 1)*

264 Stratigraphic plots of the macrophyte, diatom and invertebrate functional groups were  
265 made using the “Rioja” Package in R (Juggins, 2009). Major zones of change were  
266 determined through “coniss” clustering analysis. The relative abundance of the  
267 macrophyte, diatom and invertebrate species functional groups was log transformed  
268 ( $\log+1$ ) prior to clustering analyses to weight the varying relative abundances of the  
269 different functional groups (Legendre and Gallagher 2001).

270 To summarize the principle gradients of temporal change in the functional groups and  
271 geochemical data, we used Multiple Factor Analysis (MFA) (Pagès, 2002) in R  
272 (*Factoextra* and *FactoMiner* packages in R). This multivariate technique describes core

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273 depths by the several sets of quantitative variables clustered into the following group  
274 categories: macrophytes, diatoms, invertebrates, biomarkers, and geochemical data. The  
275 analysis takes into account the contribution of all groups of variables to define the  
276 distance between core depth samples.

**Commented [AO17]:** What distance? This isn't clear? Do you mean multi-variate space distance?

277 We ran the MFA on the palaeoecological data spanning pre-canal times to the present.  
278 To balance the number of samples among proxies, we selected 23 samples out of the 34  
279 total XRF data. These XRF samples corresponded to same sediment levels analysed for  
280 macrofossils and diatoms. Given that we could only analyse ten sediment samples for  
281 biomarkers, missing information for the time periods 2008, 2005, 2000, 1993, 1976,  
282 1972, 1923, 1900, 1871, and pre-1870 were replaced in the MFA by the mean value of  
283 each variable (Pagès, 2002). The relative abundances of the functional groups within each  
284 group category were normalized by applying a weight equal to the inverse of the first  
285 eigenvalue of the analysis of the group (Pagès, 2002).

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286 *Environmental and hydro-climatic effects (Objective 2)*

**Commented [RN19]:** See comment above

287 To quantify the role of hydro-climatic and physical-chemical variables in determining the  
288 long-term limnological change, we used MFA and multi-model ranking generalised lineal  
289 models (GLM). These analyses focused on the time-period 1972-2013 from which  
290 historical physical-chemical and hydro-climatic data were available (Table 1). We  
291 included all palaeoecological groups' data (n= 12 sediment samples for each proxy),  
292 except biomarkers due to the low number of samples. The relative abundances of the  
293 functional groups and the physical-chemical and hydro-climate data within each group  
294 category were again normalized in the MFA by applying a weight equal to the inverse of  
295 the first eigenvalue of the analysis of the group.

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**Commented [RN21]:** Linear?

296 For GLMs, prior to analysis we summarised the macrophyte, diatom, invertebrate and  
297 the XRF temporal change (turnover) through a single response gradient via principal  
298 curve (PC) analysis (prcurve in R; De'ath, 1999). By using nonlinear regressions and  
299 smoothers, the PC ordination analysis extracts a single gradient from each of the  
300 macrophyte, diatom and invertebrate species data and the geochemical data by passing  
301 through the multivariate ordination space (De'ath 1999). PC analyses were run on each of  
302 the biological and geochemical groups using Canonical Analysis as the starting point on  
303 *log+1* transformed data.

304 To construct a parsimonious model, we then selected the most influential variables of  
305 the physical-chemical and hydro-climatic data on macrophyte, diatom, invertebrate and  
306 geochemistry turnover. Variable selection was assessed via Spearman's correlation  
307 analysis (Fig. S3.1). As macrophyte turnover can play a structuring role on aquatic  
308 organisms and lake geochemistry (Jeppesen et al., 1998), we included the macrophyte PC  
309 values as an explanatory variable for the GLM analysis of diatoms, invertebrates and  
310 geochemistry.

311 Multi-model ranking were assessed using the “MuMIn” package in R (Bartoń, 2016)  
312 according to three logical steps: (i) we first ran a global GLM model including all the  
313 variables of interest; (ii) we then used the function dredge() to assess all possible models  
314 with different combinations of structural variables. For each model, we included additive  
315 and interaction terms; and (iii) we selected the best models according to Akaike's  
316 Information Criterion (AIC), differences for the comparison to the lowest AIC value  
317 ( $\Delta$ AIC) and the AIC weight (AICw= probability of being the “best” model) (Feld et al.,  
318 2016). The final model outputs were restricted by the lowest AIC values and pre-defined

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Ranking was?

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319 thresholds of  $\Delta AIC \leq 2$  and cumulative  $AICw \leq 0.95$  (Feld et al., 2016). The performance  
320 of the selected best models was then assessed according to goodness-of-fit (adjusted  $R^2$ ).  
321 The significance of important explanatory variables in each model was assessed via  
322 ANOVA.

## 323 **RESULTS**

### 324 **Core chronology and sedimentation rates**

325 Due to relatively fast sedimentation rates, it was difficult to estimate the equilibrium  
326 depth of total  $^{210}\text{Pb}$  activity with supported  $^{210}\text{Pb}$  in the core (Fig. 2a). Given the non-  
327 monotonic variation in unsupported  $^{210}\text{Pb}$  activities (Fig. 2b), chronologies were  
328 calculated using the CRS dating model (Appleby et al., 1986) and confirmed by the  $^{137}\text{Cs}$   
329 activities record (detectable between 29-44 cm of core depth; Figure 2c). The  $^{210}\text{Pb}$  CRS  
330 model resulted in a chronology spanning the last c.150 years (Fig. 2d). Pre-canal riverine  
331 conditions were contained within the 87-50 cm section of the core and the post-canal lake  
332 conditions within the top 50 cm. The age model showed that sedimentation rates at post-  
333 canal times remained relatively uniform (each cm representing c. 8-15 years) until the  
334 early-2000s when rates almost doubled (Fig. 2).

### 335 **Limnological evolution of the study basin**

336 Temporal variation of the selected geochemical and biomarker data is presented in Fig. 3.  
337 Sixteen macrophytes, 19 invertebrates and 81 diatom taxa were identified throughout the  
338 sediment core (Table S1) and the selected functional biological groups are presented in  
339 Fig. 4. The MFA on the palaeo-data showed that the first two dimensions explained

340 47.4% of the total variation (Fig. 5a). The first dimension was linked to the variation in  
341 all three biological groups and geochemical elements, while the second dimension was  
342 mainly associated with shifts in macrophytes, biomarkers and diatoms. Cluster analysis  
343 revealed four distinct temporal zones of biological and geochemical change (Figs, 3,4)  
344 and represented in the MFA as follows:

345 *Zone 1* (c. pre-1870)

346 Samples of this zone were placed on the lower right hand side of the MFA ordination plot  
347 (Fig. 5a); this zone is characterized by enhanced concentrations of K and Fe/Mn and  
348 Ti/Ca elemental ratios. Higher GDGTs MI index values and relatively high contributions  
349 of bacteria-algae derived *n*-alkanes were also observed. Tychoplanktonic (e.g.  
350 *Pseudostaurosira brevistriata*, *Fragilaria mesolepta*) and benthic freshwater (e.g.  
351 *Brachysira microcephala*, *Nitzschia amphibia*, *Achnanthydium minutissimum* and *A.*  
352 *exigum*) diatom taxa prevailed (Fig. S1.3). Freshwater planktonic diatoms (e.g.  
353 *Aulacoseira granulata*, *Discostella stelligera*) were also present. Emergent rushes  
354 (*Juncus* spp.) and sedges (*Carex* spp.) were abundant along with submerged charophytes  
355 (*Chara* spp. and *Nitella* spp.), anchored-floating water lilies (likely *Nymphaea ampla*),  
356 and free-floating plant species such as *Ludwigia sedoides*, *L. helminthorrhiza*, *Pistia*  
357 *stratiotes*, *Salvinia rotundifolia* and *Eichhornia* spp. (Fig. 5a, Fig. S1.1). Invertebrates  
358 associated with macrophyte-detrital habitats were abundant with *Cladopelma* spp.,  
359 *Zavreliella* spp., and *Stenochironomus* spp., being the most common. Filter-feeder  
360 bryozoans (*Lophopodella carteri* and *Plumatella* spp.) and Trichoptera shredders were  
361 also present (Fig. 4, Fig. S1.2).

362           Zone 2 Canal works (c.1871-1913)

363   This zone moved towards the upper right hand side of the MFA plot presenting Pb, Zn,  
364   and Cu increased concentrations along with relative abundances of macrophyte-derived  
365   *n*-alkanes (Fig. 5a). The macrophyte charophytes, rushes and sedges disappeared, while  
366   anchored-floating plants (particularly *N. ampla*) increased (Fig. S1.1). Planktonic (e.g.  
367   *Aulacoseira granulata* and *Aulacoseira* cf. *alpigena*) and benthic freshwater diatom  
368   species (e.g. *N. amphibia*, *Navicula radiosa* and *Navicula recens*) became more abundant  
369   and diverse (*Melosira linearis* appears for the first time), followed by benthic species  
370   (e.g. *Brachysira microcephala*, *Encyonema minutum*, *Encyonema silesiacum*, *Pinnularia*  
371   *major*, *Pinnularia subcapitata*) normally associated with nutrient-poor, and acidic  
372   conditions amongst neotropical swampy environments (Ref) and to a lesser degree  
373   *Nitzschia amphibia* normally associated with nutrient-rich conditions (Velez et al., 2013)  
374   (Figs. 5a, S1.3). The chironomids *Cladopelma* spp., *Zavreliella* spp., and  
375   *Stenochironomus* spp. declined while the bryozoan filter-feeding (*L. carteri*, and  
376   *Plumatella* spp.) and Trichoptera shredders increased. The non-indigenous bryozoan *A.*  
377   *gelatinosa* appeared for the first time in this Zone (Fig. 4, Fig. S1.2).

378           Zone 3 Gatun Dam (1923-1994)

379   During this zone, the study samples moved towards the lower left hand side of the MFA  
380   plot in response to a decline in the concentrations of K, Pb, Zn, and Cu and Fe/Mn and  
381   Ti/Ca elemental ratios (Figs. 3,5a). The *n*-alkane TAR index also increased along with  
382   the contribution of terrestrial plant-derived *n*-alkanes. There were progressive increases in  
383   submerged macrophytes (e.g. *Najas guadalupensis*, *Najas marina*, and *Ceratophyllum*

Commented [RN24]: Representing?

384 *demersum*) and in free-floating plants (e.g. *Eichhornia* spp., *L. sedoides/helminthorrhiza*,  
385 and *P. stratiotes*) (Fig. 4, Fig. S1.1). The invasive species *H. verticillata* colonised the  
386 system while anchored-floating plants gradually declined. Benthic aerophilous diatom  
387 species increase (e.g. *Diadsmis*, *Luticola* and *Orthoseira*), as well as benthic species  
388 from nutrient-rich environments (e.g. *Cocconeis placentula*, *Planothidium lanceolatum*)  
389 (Fig. 3, Fig. S1.3). Planktonic *A. granulata* increase slightly. The abundances of most  
390 pre-canal diatom taxa declined while salinity-tolerant species (*Terpsinoe musica*, and  
391 *Tabularia fasciculata*) increased. Invertebrate shredders, filter-feeding and  
392 macrophyte/detrital associated taxa declined gradually, while benthic chironomids  
393 (*Coelotanypus* spp.) increased (Fig. 4, Fig. S1.2).

#### 394 *Zone 4* Recent changes (1995-2013)

395 During this zone, the study samples moved towards the upper left hand side of the MFA  
396 plot reflecting the increases in calcium (up to four times historical values; Fig. 3), in the  
397 ratio between aquatic and terrestrial plant inputs (Pmar-aq), and in declines in *n*-alkane  
398 TAR index. A second peak in the GDGT MI index at 2010-2011 was also detected (Fig.  
399 3). Progressive increases in the contribution of algae-derived and bacteria-derived *n*-  
400 alkane were further observed post-2006. All four of the macrophyte growth-types steadily  
401 increased during this zone (Fig. 4, Fig. S1.1). Diatom functional groups of Zone 3  
402 prevailed but with increases in benthic, aerophilous *Diadsmis confervacea* and benthic,  
403 *Eunotia incisa*, *E. monodon* and the salinity-tolerant species *T. musica* (Fig. 4, Fig. S1.3).  
404 After the 1990s benthic diatoms from nutrient-rich environments dominated (mainly *C.*  
405 *placentula clevei*, *Gomphonema grovei* and *N. amphibia* cf. *Mayamaea*). Aerophilous

Commented [RN25]: Elsewhere just Ca

406 and benthic species tolerant of salinity increased. Grazers (daphnids, molluscs and the  
407 chironomid *C. plumosus*) and benthic invertebrates dominated while both bryozoan filter-  
408 feeding and Trichoptera shredders declined (Fig. 4, Fig. S1.2).

#### 409 **Environmental and hydro-climatic effects**

410 Dissolved oxygen (DO) at the water surface, and conductivity emerged as collinear  
411 variables (Tables S2.1, S2.2), hence we exclude these two variables from the MFA and  
412 GLM analyses. We also excluded pH as it was strongly related with secchi depth  
413 (positively) and precipitation (negatively; Figure S3.1).

414 The MFA on the selected palaeo-data (macrophytes, diatoms, invertebrates and  
415 geochemical parameters) and historical abiotic (hydro-climatic and physical-chemical)  
416 parameters explained 52% of the total variation (Fig. 5b). The first dimension was  
417 positively linked with the hydro-climatic variables (flow and precipitation), Ca,  
418 sedimentation, submerged and both floating macrophyte groups, grazers and benthic-rich  
419 diatoms. Filter-feeding, shredders, and macrophyte/detrital invertebrates, planktonic and  
420 salinity tolerant diatom species, secchi depths and phosphorus, and Ti/Ca and Fe/Mn  
421 were in turn, negatively related with dimension 1. The second dimension was positively  
422 associated with DO, littoral diatoms, and emergent plants, and negatively related with  
423 Chl-a, periphytic and aerophilous diatoms, and anchored-floating and submerged  
424 macrophytes.

425 Spearman correlation highlighted precipitation (3-year average), secchi depth and  
426 Chl-a were the most important predictors of macrophyte species turnover (Fig. S3.1;  
427 Table 2). The combined GLM multi-stressor model resulted in three possible models with  
428 the greatest goodness-of-fit model explaining 85% of the total adjusted macrophyte

429 species turnover. Precipitation alone explained a significant ( $P < 0.001$ ) 53% of  
430 macrophyte species turnover, Chl-a 22% ( $P < 0.001$ ) and secchi depth 6% ( $P < 0.05$ ). The  
431 interactions between Chl-a and precipitation ( $P < 0.05$ ) and between **Secchi** and Chl-a  
432 ( $P < 0.05$ ) explained 6% and 5% of macrophyte species turnover respectively.

**Commented [RN26]:** Elsewhere not capitalized – choose one.

433 For diatom species, Spearman correlation identified turnover, annual flow,  
434 macrophyte turnover, and phosphorus as the most important predictors of diatom  
435 turnover (Fig. S3.1; Table 2). The combined GLM multi-stressor model resulted in two  
436 best models with the greatest goodness-of-fit model reflecting only the single effects of  
437 plant cover and phosphorus, and the interaction between plant turnover and phosphorus.  
438 The model explained 67% of the adjusted variation in diatom species turnover.

439 For invertebrates, Spearman correlation identified annual flow, secchi depth and  
440 phosphorus as the best explanatory predictors of the invertebrate species turnover (Table  
441 2). The multi-stressor analysis resulted in **five best models**. The model with the greatest  
442 goodness-of-fit explained 63% of the adjusted variation in invertebrate species turnover.  
443 Annual flow explained a significant ( $P < 0.01$ ) 35.7%, secchi depth ( $P < 0.01$ ) 17%, P  
444 ( $P < 0.05$ ) 12%, whereas the interaction between secchi depth and phosphorus explained a  
445 non-significant 12% of the adjusted variation in invertebrate species turnover.

**Commented [RN27]:** Can you have 5 best things? Were they all equally good?

446 Spearman correlation resulted in annual flow, DO in the water column,  
447 sedimentation rates and the interaction between DO and sedimentation rates as the best  
448 predictors of geochemical variables turnover (Table 2). The multi-stressor analysis  
449 resulted in **one best model** explaining 92% of the adjusted variation in geochemical  
450 variables turnover. The model included only the effects of sedimentation rates ( $P < 0.001$ )

**Commented [RN28]:** As above  
The best model?

451 62%, DO 23.6% ( $P < 0.001$ ) and the interaction between DO and sedimentation rates 5%  
452 ( $P < 0.01$ ).

## 453 **DISCUSSION**

454 Our data uncover a dynamic limnological history of the study lake basin. Our records  
455 begin before the formation of Gatun Dam by the Panama Canal and extend to the present  
456 day, clearly describing the expected gradual transition from a river-governed swampy  
457 environment to a lake setting. Before the creation of the canal, the Chagres River  
458 meandered through an alluvial floodplain of vast areas of swamp (Albrecht et al., 2017,  
459 McCullough, 2001) and this is corroborated by our data. Geochemical and biomarker  
460 data indicate swamp conditions by identifying reductive sediments, low nutrient and  
461 acidic waters, and high detrital inputs (Fig. 5a). We also find a prevalence of rushes and  
462 sedges, free-floating plant species, tycho planktonic and benthic diatoms (e.g. *Fragilaria*  
463 spp., *Eunotia* spp., *Encyonema* spp. and *Pinnularia* spp.) and macro algae charophytes,  
464 all of which commonly occur in low turbulence and low nutrient waters (Fayó et al.,  
465 2018; Kolada et al., 2014, Montoya-Moreno et al., 2013). The occurrence of planktonic  
466 diatoms, in particular *A. granulata* species, which have been previously associated with  
467 riverine conditions in the subtropics and neotropics (Velez et al., 2012, Jaramillo et al.,  
468 2017, Fayó et al., 2018, Gell and Reid, 2014), further suggest an environment that was  
469 hydrologically-connected to a main river channel. Abundant Trichoptera shredders and  
470 macrophyte/detrital-associated invertebrates, along with anaerobic bacteria-archaea, all  
471 suggest a littoral and highly reductive environment. In particular, the latter suggests that,

472 at the time, carbon cycling in the study basin might have occurred mainly through  
473 methanogenesis and sulphate reduction pathways (Tabacchi et al., 1998).

474 Our results reveal a clear and major anthropogenic signal associated with the  
475 construction of the Panama Canal. Pollutants typically associated with mining and fossil-  
476 fuel combustion (Miller et al., 2014) such as Cu, Zn, and Pb increased considerably from  
477 c.1870-1913 (Fig. 3). This was most likely caused by highly-elevated coal combustion  
478 from the intensive excavation and dredging of the canal (McCullough, 2001).

479 During the same time period, our results reveal the transformation of swamps to  
480 shallow lentic environments as the area became flooded. Lake-associated taxa, such as  
481 submerged and anchored-floating plants, and shredding and filter-feeding invertebrates  
482 increased. Diatom communities shifted from tychoplanktonic to benthic-planktonic. The  
483 bryozoans *L. carteri* and the colonizing *A. gelatinosa* increased, likely because of an  
484 expansion of their submerged and floating macrophyte habitat (Ricciardi and Reiswig,  
485 1994; Wood and Okamura, 1998; Ricciardi and Reiswig, 1994). Finally, caddisfly larvae  
486 proliferated, potentially driven by increased food availability as they prey on bryozoans  
487 (Ricciardi and Reiswig, 1994), or driven by increases in littoral detritus (Ti/Ca) from the  
488 recently flooded forest and/or expanding aquatic vegetation (Sousa et al., 2011)

489 In 1913 the lake basin was flooded by the construction of the Dam. At this time we  
490 observed a decline in littoral detritus (Ti/Ca) and erosion river-fed elements (e.g. K).  
491 Such a reduction in erosion may have been caused by stabilisation of the soils when  
492 excavations slowed. Post-dam erosion likely declined further with the 1935 construction  
493 of the Alajuela Dam in the headwaters of the Chagres, reducing the supply of river  
494 material into Gatun Lake (Loewenberg, 1999). However, from 1913? until the mid-1980s

**Commented [JS29]:** what is the evidence for why K is a riverine source?

**Commented [RN30]:** Construction?



495 allochthonous organic carbon contributions increased in the lake basin. This terrestrial  
496 carbon may have come from flooded forest areas or from particulate material derived  
497 from deforestation that took place in the watershed following dam construction, and  
498 which peaked during the mid-1970s (Wadsworth, 1978). Increasing terrestrial organic  
499 matter inputs from early-1920s to the mid-1980s partially support recent findings  
500 showing that the degradation of flooded forest material in tropical impoundment projects  
501 may endure for a couple of decades after reservoir infilling, a period when CO<sub>2</sub> and CH<sub>4</sub>  
502 production is commonly facilitated (Tranvik et al., 2009; Campo and Sancholuz, 1998).  
503 Yet, such carbon pathways may take even longer to develop (four-five decades), for some  
504 lake areas, than previously suggested for tropical dam projects (Tranvik et al., 2009;  
505 Campo and Sancholuz, 1998).

Commented [AO31]: Nice!

506 Our findings show that submerged and floating macrophyte growth was encouraged  
507 in the basin as Gatun Lake infilled from 19XX, promoting aerobic conditions (Figs. 2, 3).  
508 This trend mirrors historical records of macrophyte abundance in Gatun Lake (Von  
509 Chong 1986; ACP, 2012), and corroborates patterns observed in other similar tropical  
510 impoundment projects (Agostinho et al., 1999; Scheffer et al., 2003). For instance, the  
511 invasion of *H. verticillata* that resulted in many hectares of the lake becoming choked  
512 with this submerged species (von Chong, 1986) was accompanied by increases in other  
513 submerged plants such as *N. marina* and *N. guadalupensis* and *C. demersum*. Free-  
514 floating plants, such as *Eichhornia* spp., and *P. stratiotes*, also dispersed rapidly, while  
515 anchored plants (*N. alba* in particular) gradually diminished.

516 Following impoundment and flooding diatom and invertebrate changes mirrored  
517 trends in macrophytes. Over time, invertebrate communities shifted in the lake basin from

518 detrital to benthic associations, while filter-feeding bryozoans declined, perhaps in  
519 response to the gradual decline of their principle habitat; anchored-floating plants  
520 (Ricciardi and Reisinger, 1994). The shift to a benthic-aerophilous diatom assemblage  
521 suggests that the progressive littoral macrophyte expansion provided an increase in  
522 habitat availability for benthic species and suitable littoral habitats for aerophilous species  
523 (Reid et al., 2018).

524 In 1995, after more than 80 years of impoundment, submerged and free-floating  
525 macrophytes increased in the study basin and carbon cycling shifted to within-lake  
526 production, as shown by the high Pmar-aq index value (Ficken et al., 2004). This shift in  
527 habitat structure marked an upsurge in the abundance of grazing invertebrates (molluscs  
528 in particular) and benthic diatom species associated with productive environments, a  
529 pattern previously described for shallow lakes undergoing increases in macrophyte  
530 productivity (e.g. Salgado et al., 2018; Reid et al., 2018). The increases in molluscs  
531 further concur with the deliberate introduction of the apple snail (*P. bridgesii*) across the  
532 lake to control the expanding vegetation (Angher, 1999), and the first reports of the red-  
533 rimmed melanian snail (*M. tuberculata*) in 2003 (Garcés and Garcia, 2004), which could  
534 have been benefited from the increases in epiphytic food.

### 535 **Environmental and hydro-climatic effects**

536 Our results revealed that after a century of the dam construction, natural riverine  
537 environmental drivers still exert a strong influence over the structure and functioning of  
538 the studied lake basin. In particular, hydro-climatic variables emerged as the most  
539 important factors driving the temporal variations of the abiotic and biotic compartments

Commented [JS32]: Again

540 (Fig. 4b, Table 2); in agreement with floods and droughts being major drivers of river  
541 abiotic change and community reorganization (Poff, 2002). Long-term shifts in hydro-  
542 climatic variables have been suggested to alter a series of interconnected processes such  
543 as sedimentation, water chemistry and transparency, sediment reductive conditions and  
544 primary productivity. During drier periods, for instance, sedimentation was low while  
545 physical erosion and detrital inputs increased. There was also a prevalence of reductive  
546 sediments and relatively high secchi depths (> 3m), which correlated positively with  
547 oxygenated surface waters (> 6 ppm), higher conductivity (>60  $\mu\text{S}/\text{cm}$ ) and higher  
548 nutrient availability (Table 1). Increases in conductivity resulting from reduced dilution  
549 of salt ions during the dry season have been similarly observed in the Amazon River,  
550 where conductivity in oxbow lakes can increase up to 200-times the value of the main  
551 river (Junk et al., 1989). Accumulation of organic matter and debris in the lake bed  
552 causing reductive soils has been also described in oxbow lakes associated with the Paraná  
553 River where they were attributed to low rates of water circulation during drier phases  
554 (Sousa et al., 2011). Anoxic sediment conditions in our study basin, would have  
555 transformed nutrients (phosphorus in particular) into available forms (Mitsch and  
556 Gosselink, 2015) that, along with clearer and stable water levels, would have favoured  
557 planktonic diatoms, filter feeding invertebrates, submerged and free-floating macrophytes  
558 and Chl-a (Junk et al., 1989; Sousa et al., 2011), ?all of which we observe?. As  
559 submerged plants grow in clearer waters rates of photosynthesis would also increase,  
560 raising DO levels in surface waters (Spence and Chrystal, 1970).

561 Wet periods were linked to increases in sedimentation, deposition of minerals (e.g.  
562 Ca and K) and reduced water transparency (Fig. 4b). Lower secchi depths were, in turn,

**Commented [JS33]:** why sedimentation is low while physical erosion is high?

**Commented [AO34R33]:** This is confusing to me too

563 associated with lower values of pH, conductivity and dissolved oxygen, and this likely  
564 reflects nutrient inputs and storage of organic matter during the dry periods, derived from  
565 both autochthonous production and allochthonous inputs from the diverse and? lavish  
566 riparian vegetation (Sousa et al., 2011). These changes in physical-chemical conditions  
567 could have expanded available ecological niches thus potentially explaining the increase  
568 in primary producer diversity (Table 2; Junk et al., 1989; Sousa et al., 2011). Increases in  
569 freshwater discharge would have further promoted propagule dispersion, especially for  
570 macrophytes (Sousa et al., 2011), whereas light attenuation in the water column coupled  
571 with fluctuating water levels would have stressed the submerged vegetation while  
572 favouring floating macrophyte growth through nutrient inputs from flooded land (Junk et  
573 al., 1989).?All of which mirror patterns observed?

**Commented [JS35]:** would thi changes stimulate primary producers or just diversity among porducers?

574 Paleocological data has many limitations and can suffer from biases (Zhao et al.  
575 2001, Clarke et al., 2014). Uncertainties in the age model and the different historical  
576 physical-chemical sources may also introduce discrepancies in multi-variable models.  
577 Similarly, large prairie reservoirs combine numerous riverine and lacustrine environment  
578 features; hence limnological processes may present large spatial variation across the lake  
579 (Maavara et al., 2015). Thus, whether our results from a single core and a single basin  
580 fully reflect lake-wide historical changes warrants further study. Nonetheless, our  
581 multiple and independent lines of evidence of change in biotic and abiotic variables are  
582 all in general agreement. Moreover, the magnitude and timing of changes observed in our  
583 record coincide with the known basin-wide anthropogenic history of the Panama Canal as  
584 well as with known natural climatic and environmental changes in the region and with  
585 tropical riverine systems in general (e.g. Mitsch and Gosselink, 2015; Junk et al., 1989;

**Commented [RN36]:** Probably need to explain what biases – leave this in?

**Commented [RN37]:** I wonder if this could be removed as you address the point about a single core very well at the end of this section.

Don't want to overdo it!

586 Sousa et al., 2011). Our plant macrofossil data for instance, concur with the main  
587 vegetation changes previously described for the lake (Von Chong, 1986). A coherent  
588 signal in declines in Ca coupled with increases in sedimentation rates, Fe/Mn, and Ti/Ca  
589 during 2010-2011 (Fig. 2) further resemble the riverine pre-damming conditions and  
590 matched with the recent “La Purísima” rainstorm in 2010, which flooded the whole lake  
591 system and increased sedimentation rates almost 100-fold (ACP, 2014). Multi-coring  
592 palaeoecological studies in shallow lakes having a relatively similar size to our study  
593 basin (e.g. Sayer et al., 2010; Salgado et al., 2018) have also indicated that despite some  
594 variation in the distribution of certain aquatic species amongst lake areas, the  
595 palaeoecological signal across cores reflect a coherent trend of whole lake change  
596 dynamics. Thus, we are confident that despite our single core approach, our data are  
597 reflective of a general historical change at the basin scale.

#### 598 **Is the Gatun Lake becoming more saline?**

599 Our records suggest a gradual increase in salinity in our study basin. From the mid-1960s  
600 to mid-1980s a salinity-tolerant diatom species is observed (Figs. 3, 5b), while from the  
601 early 2000s to the present day we observe an increase in both saline-tolerant diatoms  
602 (including two marine morph types; Fig. S3f) and Ca concentrations. These early  
603 increases are likely caused by runoff into the enclosed basin resulting in increased ion  
604 input and hence increased water salinity. Nevertheless, while greater runoff is often the  
605 principle driver of ion input, evaporation can, in some cases, also increase salinity by  
606 ????? (Mitsch and Gosselink, 2015). This may explain the observed peak in the salinity-

607 tolerant diatom from mid-1960s to mid-1980s which was a period of relatively low  
608 precipitation (Figs. 3, 5b).

609 In 2005 the Panama Canal began a major expansion to deal with predictions of  
610 increasing global trade (Wijsman, 2013), dredging and excavations may have increased  
611 ion concentrations into the lake, thus explaining increasing Ca and salt-water tolerant  
612 diatoms in the lake. Expansion of the canal culminated with the opening of a new set of  
613 larger locks that use a tiered water sharing system with the potential to move saltwater  
614 from the sea up into the lake more easily compared to the old locks. Our records predate  
615 the opening of these new locks in 2016, but unpublished records suggest the lake may be  
616 increasing in salinity faster than before the opening of the new locks.

617 It is therefore difficult to predict future salinity changes in the lake, but the historical  
618 records we present here provide crucial baseline data. Irrespective of the impact of the  
619 new locks, salinity in the lake may increase, especially if deforestation is stepped up or  
620 evaporation is enhanced by global climate change (as predicted by Engelbrecht et al.,  
621 2007). Nevertheless, given that these “background” drivers of increasing salinity have  
622 caused only relatively minor increases over decades, these processes could be dwarfed by  
623 salt intrusions from the new locks. Either way, the implications for increasing salinity of  
624 the canal are extremely concerning and have yet to be fully explored. Drinking water  
625 (Condit et al., 2001) and the environment and ecology of the lake itself may be  
626 threatened, and the loss of the freshwater barrier to intercontinental dispersals (Ros et al.)  
627 could be catastrophic. In addition, the recent increase in *n*-alkane bacteria and both the  
628 MI and Pmar-aq indices that we observe could be a warning that the halocline will render  
629 eventually surface sediments anoxic (Mead et al., 2005), as has occurred here or here.

Commented [RN38]: Accelerated?

Commented [AO39]: M. Ros, G. V. Ashton, M. B. Lacerda, J. T. Carlton, M. Vázquez-Luis, J. M. Guerra-García, G. M. Ruiz. The Panama Canal and the transoceanic dispersal of marine invertebrates: evaluation of the introduced amphipod *Paracaprella pusilla* Mayer, 1890 in the Pacific Ocean. *Mar. Environ. Res.* **99**, 204–211 (2014)

Commented [RN40]: For...

Commented [AO41]: Not sure if you can give an example?

630 **Invasions and ecological responses**

631 Our palaeontological record is coherent with reported introductions of invasive taxa (e.g.  
632 *P. bridgesii* and *M. tuberculata*) and patterns of spread (e.g. *Eichhornia* spp.) in the  
633 Gatun Lake. We also consider the date for the first introduction of the Asian bryozoan *A.*  
634 *gelatinosa*, first reported in 1998 (citation needed), to be much earlier; in the early-1900s.  
635 This suggests that despite its exotic status, this species might not be directly competing  
636 with other native bryozoans (e.g. *L. carteri*) and instead shifts in macrophyte cover and  
637 physical-chemical conditions were the principle drivers of changes in the bryozoan  
638 communities.

639 The invasion of peacock bass in 1969 had a profound effect on the lake's native  
640 littoral planktonic fish community (Zaret and Paine, 1973) that resonates today with  
641 native populations being continually marginalised (Sharpe et al., 2017). Zaret and Paine  
642 (1973) predicted that fish predation would lead to cascading effects through the lake's  
643 food web, particularly on littoral zooplankton (e.g. *Ceriodaphnia*), aquatic insects (e.g.  
644 mosquitos/chironomids) and primary producers. Our results do not support the latter, as  
645 cladoceran ephippia only became apparent after the late-1990s, a period that instead  
646 coincides with increasing *n*-alkane algae contribution, again supporting the importance of  
647 abiotic conditions on the lake's ecosystem. We found no evidence of increasing  
648 abundances or shifts in specific functional groups (e.g. planktonic taxa or reductions in  
649 chironomid abundances) during or post-invasion times that would support evidence for a  
650 long-term cascading effect of predation down the food-web. Instead, evidence during this  
651 period points towards a bottom-up flow of energy and nutrients coupled with asymmetric

**Commented [RN42]:** See comments above about 'introduced species'. This is a better term?

**Commented [AO43]:** Add common names?

652 benthic–littoral production likely associated with the development of macrophytes  
653 (Schindler and Scheuerell, 2002).

#### 654 **Remarks and management options**

655 Our study reconstructs the biotic and abiotic dynamics of a shallow basin within Gatun  
656 Lake over the last ~150 years, providing a first approximation into the natural and  
657 anthropogenic impacts of the impoundment of the Chagres River. Species invasions,  
658 land-use changes and shipping traffic have all impacted the lake’s ecosystem, and we find  
659 evidence for all these in the palaeolimnological record. However, our multiple lines of  
660 evidence emphasize that, on a decadal scale, the study basin still retains some of its  
661 natural riverine function. Compared to other old impoundments in the tropics, Gatun  
662 Lake is unique by having high precipitation rates (annual mean > 2200 mm), large  
663 expanses of protected forest areas in the lake catchment (e.g. Barro Colorado Island and  
664 Soberania), and more importantly, low water retention times due to the continuous  
665 activities of the canal lock gates and hydropower generation (Zaret, 1984). These factors  
666 may help to reduce shifts in runoff, water pollution and maintaining the natural  
667 hydrological balance. It is, however, anticipated that climate change will modify  
668 precipitation, evapotranspiration, and runoff in the tropics (Engelbrecht et al., 2007).  
669 Thus, increasing prevalence of both drier and wetter periods could fundamentally modify  
670 the functioning of shallow basins within Gatun Lake. Drier periods will likely encourage  
671 on-going spread of submerged macrophytes and increases in salinity and nutrients via  
672 reduced dilution. Wetter periods in turn, may enhance sedimentation rates, nutrient  
673 inputs, and salt intrusions from storm surges and floating plant dominance. Basin-wide

**Commented [AO44]:** This is confusing as natural to me would be swamp, which it clearly is not. I think what you are trying to say is that natural drivers dominate abiotic changes and ecological structuring?

**Commented [RN45R44]:** Unless the core was taken from where the river channel was?



674 ecosystem monitoring is critical to improve our understanding of climate-ecological  
675 processes in the lake, especially with the increased threat of further invasive species and  
676 unclear predictions of how the region will respond to climate change. Finally, our  
677 assessments from this shallow basin suggest that to preserve the natural riverine system  
678 functioning in shallow areas of Gatun Lake, management activities must include the  
679 understanding of the interactive effects between key long-term ecosystem structural  
680 drivers such as river flow, runoff patterns and physical-chemical conditions.

681

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**Commented [AO46]:** Something like this is needed by SENACYT. Thanks!

704

705

706 **Author Contributions:** JS designed the study and collected the sediment material. JS  
707 produced and analysed the plant and invertebrate macrofossil data, CH and JC produced  
708 and analysed the biomarker data, and MV produced and analysed the diatom data. NR  
709 and HY produced and analysed the lithostratigraphic and radiometric data. JS wrote the  
710 first manuscript and all authors contributed substantially to the final version.

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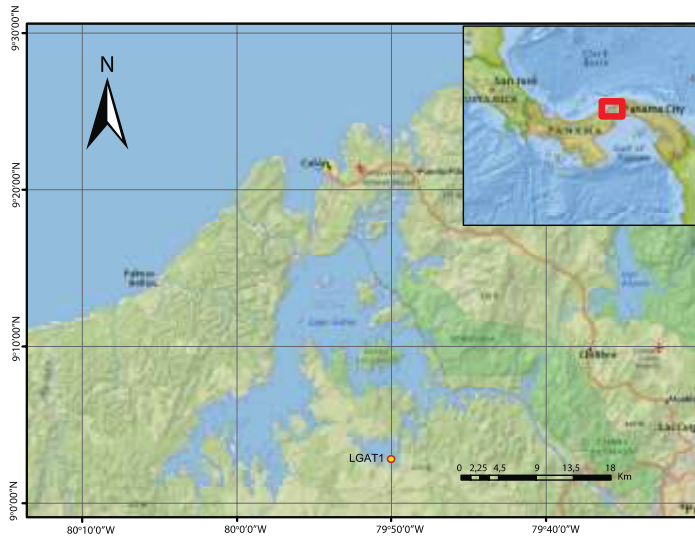
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916

917 **Figures and Tables**

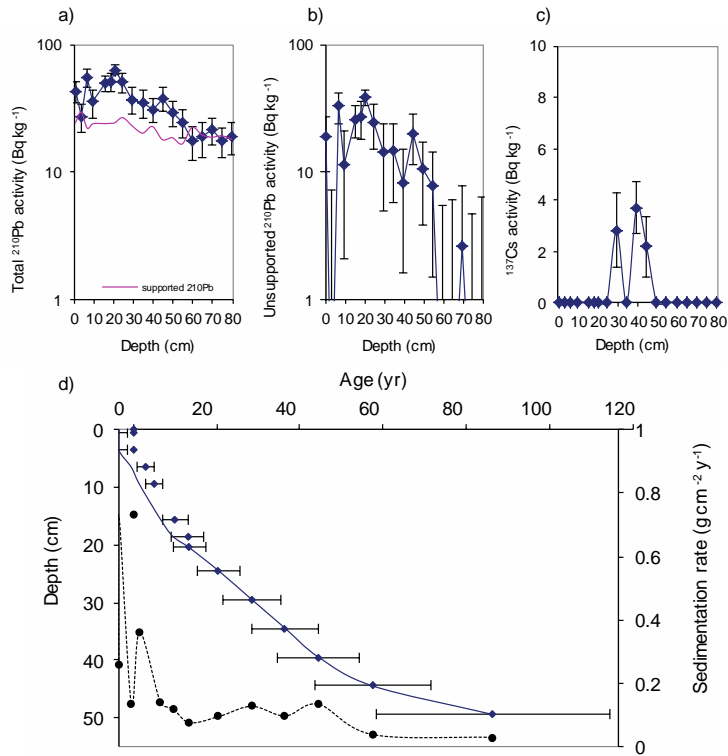


918

919 **Figure 1.** Map of the River Chagres watershed. The artificial Gatun and Alajuela lakes  
920 and the connecting River Chagres are indicated in blue. Natural protected areas are  
921 shown in dark-green. The coring location of LGAT1 core is indicated by a red-yellow  
922 circle.

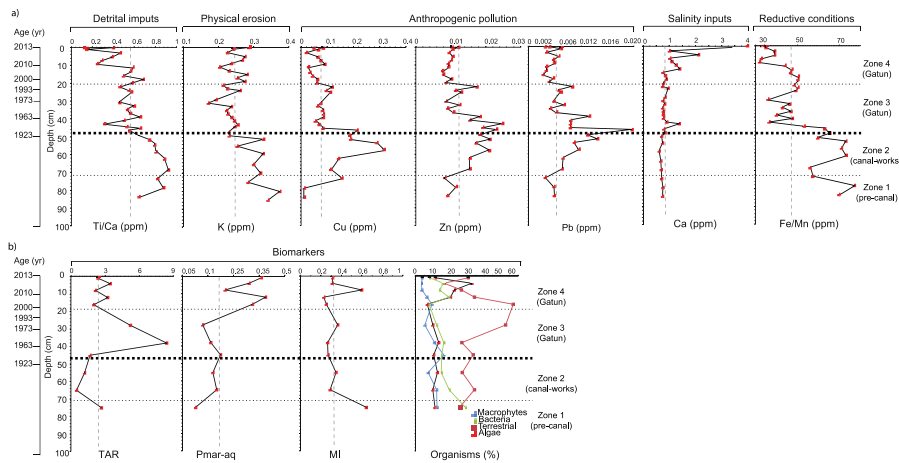
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925

926 **Figure 2.** Fallout radionuclide concentrations in core LGAT1 taken from a shallow basin  
 927 in the western area of Gatun Lake, showing (a) total  $^{210}\text{Pb}$ , (b) unsupported  $^{210}\text{Pb}$ , (c)  
 928  $^{137}\text{Cs}$  concentrations versus depth, and (d) radiometric chronology of core LGAT1,  
 929 showing the CRS model  $^{210}\text{Pb}$  dates and sedimentation rates. The solid line shows age  
 930 while the dashed line indicates sedimentation rates.



931

932 **Figure 3.** Sedimentary profile of (a) selected geochemical elements and ratios, and (b)

933 biomarkers indices in LGAT1 sedimentary core. Terrigenous aquatic ratio–TAR;

934 Methane Index–MI; the submerged/floating aquatic macrophyte inputs vs. emergent/

935 terrestrial plant input ratio–Pmar-aq. Major temporal zones of change determined by

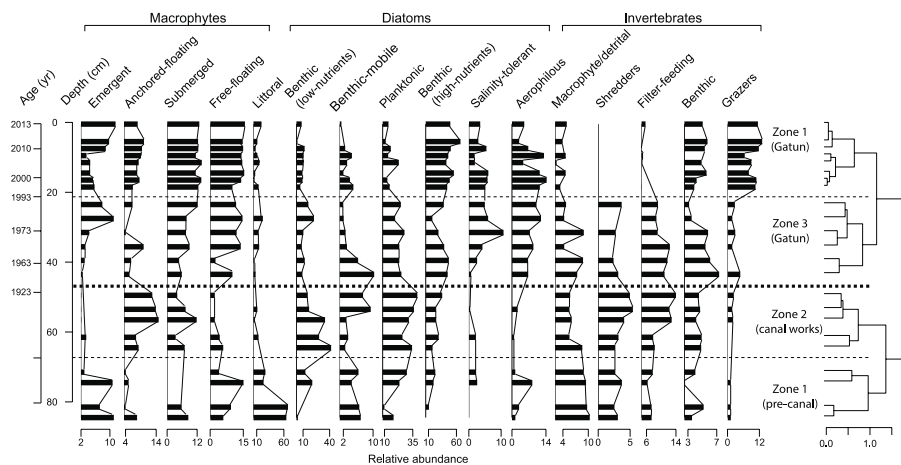
936 clustering analysis, corresponding to Zone 1 (c. pre-1870), Zone 2 (c. 1870-1914), Zone 3

937 (1923-1990), and Zone 4 (1991-2013). A vertical grey dotted line indicates the mean

938 value of each parameter.

939

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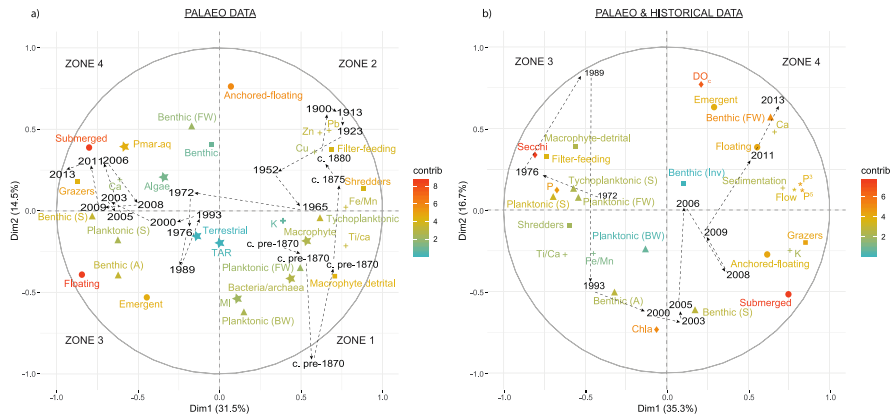


941

942 **Figure 4.** Sedimentary profile of the relative abundances of the study macrophyte, diatom  
 943 and invertebrate species functional groups in LGAT1 core. Major temporal zones of  
 944 change determined by clustering analysis are shown by dotted lines, corresponding to  
 945 Zone1 (c. pre-1870), Zone 2 (c.1870-1914), Zone 3 (1923-1990), and Zone 4 (1991-  
 946 2013). Note that the x-axis widths of each functional group are not scale according to the  
 947 relative abundance value.

948





949

950 **Figure 5.** Multiple factor analysis (MFA) plot for a) biological (macrophytes–circle,  
 951 diatoms–triangle, invertebrates–square), geochemical (Fe/Mn, Ti/Ca, Cu, Zn, K, Pb and  
 952 Ca–cross) and biomarker (MI, TAR, Pmar.aq, macrophyte, terrestrial, bacteria/archaea,  
 953 algae–star) palaeo-data for the period c. pre-canal-2013; and b) biological and  
 954 geochemical palaeo-data and historical hydro-climatic data (hash), and physical-chemical  
 955 data (diamond) for the period 1972-2013. The contribution of each variable is indicated  
 956 according to a color scale, being red the highest value and green the lowest. FW=  
 957 Freshwater, BW= brackish water, A= aerophilous, S= salinity tolerant, P<sup>3</sup>= three years  
 958 average precipitation, P<sup>5</sup>= five years average precipitation, Flow= river annual flow.  
 959 Black dash arrows indicate temporal trajectory of sediment sample change. Major  
 960 temporal zones of change determined by clustering analysis are shown corresponding to  
 961 Zone1 (c. pre-1870), Zone 2 (c.1870-1914), Zone 3 (1923-1990), and Zone 4 (1991-  
 962 2013).

963

964

965 **Table 1.** Historical data on three years average precipitation data (P<sup>3</sup>), five years average  
 966 precipitation data (P<sup>5</sup>), and annual river flow (Flow), nitrates (NO<sub>3</sub>), phosphorous (TP),  
 967 secchi depth, conductivity, pH, dissolved oxygen at the water surface (DO<sub>s</sub>) and at the  
 968 water column (DO<sub>c</sub> < 1m depth), and chlorophyll a (Chl-a) for the period 1972-2013.

Time (yrs.)	P <sup>5</sup> (mm)	P <sup>3</sup> (mm)	Flow (m <sup>3</sup> /s)	pH	DO <sub>s</sub> (mg/L)	DO <sub>c</sub> (mg/L)	NO <sub>3</sub> (mg/L)	TP (mg/L)	Cond. (µS/cm)	Chl-a (µL/L)	Secchi (cm)
2013†	2964	3381	41	6.41	6.44	5.25	0.04	0.01	42.34	2.31	146
2011†	2841	2778	38	6.35	6.60	5.12	0.07	0.01	47.86	3.22	150
2009†	2664	2640	31	6.50	6.48	5.01	0.03	0.00	49.38	4.18	167
2008†	2769	2741	31	6.59	6.28	4.23	0.03	0.01	55.62	5.09	177
2006†	2569	2685	29	6.56	6.25	3.51	0.04	0.02	55.62	2.89	212
2005†	2620	2689	27	6.53	6.23	2.52	0.03	0.01	45.21	4.72	192
2003†	2593	2802	30	7.13	6.61	2.90	0.03	0.02	50.33		
2000†	2688	2644	37	6.76	6.48	3.02	0.05		49.92		
1993§	2589	2669	28	7.39	6.22	1.94		0.04	54.00		
1989§	2517	2524	26		8.66	4.94	0.03	0.04	44.88	2.05	393
1978*	2508	2332	25	7.20	7.78		0.06		90.00	4.10	700
1972*	2331	2371	26	7.56	8.00	5.12	0.05	0.02	98.00		530

969 \*Zaret (1984); §Gutiérrez et al., 1995; † ACP (2003-2013); precipitation data were  
 970 obtained from (Steve Paton, pers. comm.) and annual flow data from ACP (2012).

971

972 **Table 2.** Generalized lineal model (GLM) analysis on the combined effects of hydro-climatic, and physical-chemical data on  
 973 macrophyte, diatom, invertebrate and geochemical turnover for the period 1972-2013. A single temporal biological and geochemical  
 974 turnover gradient was assessed through principal curve analysis prior to GLMs. As macrophyte turnover (MT) can play a structuring  
 975 role on aquatic organisms and lake geochemistry, we included the MT values as an explanatory variable for the GLM analysis of  
 976 diatoms, invertebrates and geochemistry. Model subset ranking was assessed according to Akaike's Information Criterion (AIC),  
 977 differences for the comparison to the lowest AIC value ( $\Delta$ AIC), the AIC weight (AICw=probability of being the “best” model) and  
 978 goodness-of-fit (adjusted  $R^2$ ). The best model is highlighted in bold. The significance of important explanatory variables in each  
 979 model was assessed via ANOVA under a significance level of  $P \leq 0.05^*$ ;  $P \leq 0.01^{**}$ ;  $P \leq 0.001^{***}$  and the percentage of variance  
 980 explained by each variable is showed in parenthesis.  $P^3$ =three years average precipitation,  $P^5$ =five years average precipitation,  
 981 TP=phosphorous, Sed=sedimentation rates, Chl-a=chlorophyll-a, DO=dissolved oxygen.

GROUP/MODEL	AIC	Delta AIC	Weight	$adjR^2$	Explained variance & Pr(>Chi)
<u>MACROPHYTES</u>					
<b>Chla +<math>P^3</math> + Secchi +Chla:<math>P^3</math> + <math>P^3</math>:Secchi</b>	<b>-16.3</b>	<b>0</b>	<b>0.28</b>	<b>0.85</b>	<b>Chl-a (22)<math>^{***}</math>; <math>P^3</math> (53)<math>^{***}</math>; Secchi (6)*; Chl-a:<math>P^3</math> (6)*; <math>P^3</math>:Secchi (45)*</b>
Chl-a + $P^3$ +Secchi +Chl-a: $P^3$ +Chla:Secchi + $P^3$ :Secchi	-16.1	0.21	0.253	0.84	Chl-a (22) $^{***}$ ; $P^3$ (53) $^{***}$ ; Secchi (6)*; Chl-a: $P^3$ (6)*; Chl-a:Secchi (0.2); $P^3$ :Secchi (5)*

Chl-a +P <sup>3</sup> +Secchi +Chl-a:P <sup>3</sup> +Chl-a:Secchi					Chl-a (22)***; P <sup>3</sup> (53)***; Secchi (6%)*; Chl-a:P <sup>3</sup> (6)*; Chl-a:Secchi (0.2);P <sup>3</sup> :Secchi
+P <sup>3</sup> :Secchi +Chl-a:P <sup>3</sup> :Secchi	-15.9	0.34	0.237	0.83	(5)*; Chla:P <sup>3</sup> :Secchi

DIATOMS

<b>MT +TP +MC:TP</b>	<b>14.5</b>	<b>0</b>	<b>0.762</b>	<b>0.67</b>	<b>MT (30)**; TP (35)***; MT:TP(11)*</b>
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Flow +MT +TP +MT:TP				0.62	Flow (50)**; MC (1); TP (8)*
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INVERTEBRATES

<b>Flow +Secchi +TP +Flow:TP +Secchi:TP</b>	<b>29.8</b>	<b>0</b>	<b>0.193</b>	<b>0.63</b>	<b>Flow (36)**; Secchi (17*); P (12)*; Flow:TP (3); Secchi:P (12)</b>
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Flow +Secchi +TP +Flow:Secchi	30.6	0.82	0.128	0.6	Flow (36)**; Secchi (12)*; P (12)*; Flow:Secchi (10)
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Secchi +TP	30.7	0.92	0.121	0.57	Secchi (48)*; P (17)*
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Flow + Secchi +TP +Flow:Secchi +Secchi:TP	31	1.24	0.104	0.59	Flow (36)**; Secchi (17)*; P (12); Flow:Secchi (10); Secchi:TP (3)
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Flow +Secchi +TP +flow:Secchi +flow:TP	31.5				
+Secchi:TP		1.69	0.083	0.57	Flow (36)**; Secchi (17)*; P (12); Flow:Secchi (10); Flow:TP (0.2); Secchi:TP (3)

GEOCHEMICAL

DO +Sed +DO:Sed	-23.8	0	0.94	0.92	DO(27)***; Sed (62)***; DO:Sed (5)**
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